

Original Article

Comparison of IgG ABO Antibody Titers using Conventional Tube Test and Hydrogel Medium

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Abstract:

Background: ABO antibodies are clinically important in blood transfusion practice. ABO antibody titration has been used to evaluate patient outcomes, especially in ABO-incompatible stem cell and solid organ transplantation and to assess ABO-incompatible transfusion responses. **Objective:** This study aimed to detect IgG anti-A and anti-B antibodies in group O Thai blood donors by indirect antiglobulin test (IAT) using conventional tube test (CTT) and hydrogel medium (HDM). **Materials and Methods:** Altogether, 100 serum samples obtained from group O healthy blood donors of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand were included. IgG anti-A and anti-B titers were tested by IAT using CTT and HDM without washing step. The results of antibody titers and agglutination scores were compared. **Results:** Among 100 blood donors, there were 51 males and 49 females (M:F = 1:1) and their ages ranged from 18 to 58 years. There were no association between IgG anti-A and anti-B agglutination scores and titers with age and gender. The IgG anti-A, and anti-B titers using IAT-CTT yielded higher agglutination scores than IAT-HDM ($p < 0.001$). However, a good correlation was obtained in the agglutination titers (anti-A, $r = 0.7583$ and anti-B, $r = 0.7145$). To assess the repeatability of IgG ABO antibody detection by IAT using HDM, the mean, standard deviation and coefficient of variation (CV) of 3 serum samples tested in quintuplicate. The CV of agglutination scores is within 5%. **Conclusion:** From this study, the HDM can be used to perform IAT for determination of IgG ABO antibody titers in order to eliminate washing step. Moreover, it provides reliable results and reproducible testing but the antibody titers tested by IAT-HDM were less than IAT-CTT.

Keywords : ● ABO antibodies ● Hydrogel ● Group O ● Thai

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Introduction

It is well known that ABO antibodies can cause hemolytic transfusion reactions (HTRs) and hemolytic

disease of the fetus and newborn (HDFN). Currently, ABO antibody titration has been used to evaluate patient outcomes, especially in ABO-incompatible stem cell and solid organ transplantation and to assess ABO-incompatible transfusion responses.¹⁻³ In general, IgM ABO antibodies are mostly caused immune reactions related to HTRs and organ transplant; however, IgG also plays an important role in these reactions. A previous

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study reported that IgG antibodies were responsible for poor graft outcome in ABO-incompatible kidney transplantation.⁴ Additionally, a significant adverse hemolytic event associated with high-dose intravenous immunoglobulin (IVIG) treatments, due to passive transfer of IgG anti-A and anti-B from high-titer group O donors were reported.⁵⁻⁸ Therefore, all commercial IVIG preparations have to detect levels of IgG anti-A and anti-B in the final product^{9,10} but standardization of hemagglutination testing for anti-A and anti-B is still required.^{11,12}

Routinely, IgG antibody titration can be performed by indirect antiglobulin test (IAT) using the conventional tube test (CTT) because of the cost-effective, but it is limited by difficulties in automation and standardization.¹³⁻¹⁵ In addition, other disadvantage of the CTT include the interpretation of the agglutination reaction requires skilled technicians, especially when the reaction is weak and inappropriate washing of RBCs can cause false-negative results. At present, various tests for IAT have been implemented such as gel test, enzyme immunoassay and flow cytometry and the IAT by the gel test is recommended to standardize ABO antibody titration at different institutions.¹⁶⁻¹⁸

Because the IAT by CTT is a standard test in blood bank laboratories, to reduce washing step of the IAT phase for IgG antibody detection, an aqueous gel chromatography or hydrogel medium (HDM) has been established. After a rapid single-step centrifugation, red blood cells (RBCs) attached with IgG antibodies were separated from the reaction mixture by passing through the HDM, while trace protein in the pellet did not neutralized the antihuman globulin reagent added after separation.¹⁹ This study aimed to detect IgG anti-A and anti-B antibodies in group O Thai blood donors by IAT using CTT and HDM.

Materials and Methods

Subjects

Altogether, 100 blood samples obtained from group O Thai blood donors of the National Blood Centre, Thai

Red Cross Society, Bangkok, Thailand were included in this study. They comprised 51 males and 49 females (M: F = 1:1) and their ages ranged from 18 to 58 years. Informed consent was obtained from each subject. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand. Two milliliters of each serum sample were separated from clotted blood within 24-hour after collection and kept at -80°C until use.

Methods

ABO blood group was determined by cell and serum grouping according to methods previously described.²⁰ Anti-A and anti-B antiserum for cell grouping were obtained from the National Blood Centre, Thai Red Cross Society and pooled cells for serum grouping were prepared in-house. Before the IAT, to destroy IgM antibodies in their sera, the sera were treated with 0.01M dithiothreitol for 45 min at 37°C.²¹

IgG anti-A and anti-B agglutination titers determined by IAT using CTT and HDM

Doubly serial two-fold dilutions of each serum sample were performed. Each serum sample was tested simultaneously for IgG titers of anti-A and anti-B by IAT using CTT and HDM.^{19,21} To reduce the inter-technician variation, one technician performed ABO antibody titers by CTT; while, another one performed the test using HDM. Both tests were done in parallel. For the CTT, 2 drops of each serum sample was mixed with 1 drop of 2-5% RBC suspension and incubated at 37°C for 30 min. Thereafter, the reaction mixture was washed 3 times manually with normal saline; the final wash was completely decanted. Two drops of antihuman globulin reagent (CE-Immunodiagnostika GmbH, Germany) were then added and mixed well, and the cell suspension was centrifuged and examined for agglutination of the RBCs. All reactions were read macroscopically, and negative or weak agglutination reactions were examined under the microscope (x10). To ensure quality control, the validity of negative tests was further confirmed by IgG-coated RBCs.

Finally, the IAT reaction strengths of each sample were recorded.²¹ For the IAT using HDM, following the incubation phase of RBCs and serum, then the mixture was placed in the HDM (Souzhou Institute of Biomedical Engineering and Technology Chinese Academy of Sciences, Souzhou, China). After centrifugation, RBCs passed through the water-based adhesive to the bottom of the tube, while nonspecific unbound globulin remained in the upper water-based-gel. Thereafter, the reaction solution is completely decanted; an antihuman globulin was added, centri-

fuged and examined for agglutination reactions (Figure 1). The grading of the agglutination reactions of both techniques were scored as 12, 10, 8, 5, 3, 0 for 4+, 3+, 2+, 1+, w+ and negative, respectively.²² A titer was determined as the highest dilution showing w+ agglutination. The antibody agglutination scores were calculated from the summation of scored agglutination reaction results in each dilution. To increase the validity and reliability of the evaluation, the laboratory technicians were blinded for test results.

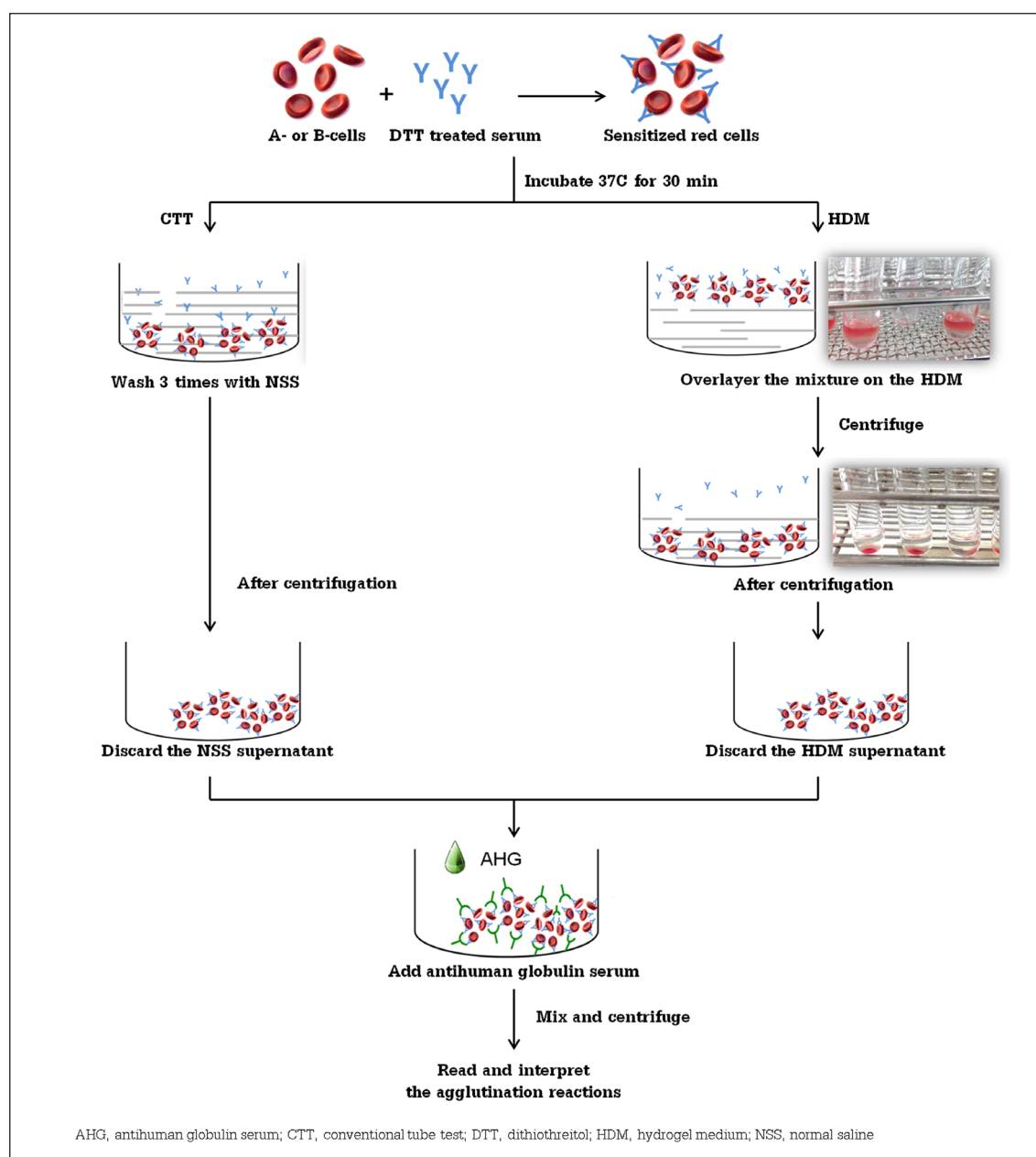


Figure 1 A schematic representation of IgG ABO antibody detection by indirect antiglobulin test using conventional tube test and hydrogel medium

Reproducibility testing for IAT using HDM

The reproducibility of IgG anti-A and anti-B titers determined by IAT using HDM was performed using randomly 3 selected group O serum samples. Each test was performed in quintuplicate.

Statistical analysis

IgG anti-A and anti-B titers obtained by IAT using CTT and HDM were compared. The Kruskal-Wallis and Mann-Whitney tests were used to evaluate the distribution of antibody titers according to age and gender. The Wilcoxon signed rank test was used to compare antibody titers among IAT-CTT and IAT-HDM. Pearson's correlation was used to analyze IgG anti-A and anti-B titers between the 2 techniques. Moreover, reproducibility of IgG anti-A and anti-B titers using IAT-HDM was evaluated by analysis of standard deviation (SD) and coefficient of variation (CV). The analysis was performed using SPSS (Version 15.0, SPSS Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Results

Distribution of IgG anti-A and anti-B antibody agglutination scores and titers according to age and gender

A total of 100 group O serum samples were tested for IgG anti-A, and anti-B titers by IAT using CTT and

HDM. The distribution of scores and titers according to different age groups (≤ 30 , 31-39, 40-49, and ≥ 50 years) was analyzed. For both IgG anti-A and anti-B, median scores and titers between IAT-CTT and IAT-HDM among different age ranges showed no significant difference ($p > 0.05$), as shown in Table 1. Moreover, the median scores and titers of IgG anti-A and anti-B between male and female donors showed no significant difference ($p > 0.05$), as shown in Table 2. The IgG anti-A and anti-B agglutination scores and titers tested by IAT was found to be significantly higher in the IAT-CTT than in the IAT-HDM for both IgG anti-A and anti-B ($p < 0.05$).

Comparison of IgG ABO antibody agglutination scores and titers by IAT between using CTT and HDM

The median (range) values of IgG anti-A agglutination scores in 100 group O blood donors determined by IAT using CTT and HDM were 60 (16-118) and 22 (3-65) and IgG anti-B agglutination scores were 61.5 (3-112) and 23 (3-97), respectively. In addition, IgG anti-A titers obtained by IAT-CTT and IAT-HDM were 128 (4-4096) and 8 (1-128) and IgG anti-B titers were 192 (1-2048) and 8 (1-1024), respectively.

Correlation of IgG ABO antibody agglutination scores and titers between by IAT using CTT and HDM

The correlation of IgG anti-A and anti-B agglutination scores and titers in 100 group O Thai blood

Table 1 Distribution of IgG anti-A and anti-B antibody agglutination scores and titers in Thai group O blood donors according to age groups (N = 100)

| IgG ABO antibodies | Age (years) | Number | Median (range) of | | | |
|--------------------|-------------|--------|-------------------------------|--------------|-----------------|------------|
| | | | Antibody agglutination scores | | Antibody titers | |
| | | | IAT-CTT | IAT-HDM | IAT-CTT | IAT-HDM |
| Anti-A | ≤ 29 | 30 | 61.5 (23-103) | 26.5 (5-64) | 128 (16-4096) | 8 (1-128) |
| | 30-39 | 31 | 60.0 (16-108) | 23.0 (3-65) | 128 (4-4096) | 8 (1-128) |
| | 40-49 | 26 | 59.0 (16-118) | 22.0 (13-57) | 128 (8-2048) | 4 (2-64) |
| | ≥ 50 | 12 | 62.0 (19-101) | 18.0 (5-57) | 256 (8-2048) | 4 (1-32) |
| Anti-B | ≤ 29 | 30 | 61.0 (34-101) | 24.0 (3-70) | 128 (16-2048) | 8 (1-128) |
| | 30-39 | 31 | 69.0 (21-96) | 23.0 (3-97) | 256 (8-2048) | 8 (1-1024) |
| | 40-49 | 26 | 59.0 (19-112) | 21.0 (3-50) | 128 (8-2048) | 4 (1-32) |
| | ≥ 50 | 12 | 74.0 (3-108) | 29.0 (3-65) | 512 (1-2048) | 8 (1-128) |

IAT, indirect antiglobulin test; CTT, conventional tube test; HDM, hydrogel medium

Table 2 Distribution of IgG ABO antibodies agglutination scores and titers according to gender in Thai group O blood donors (N = 100)

| IgG ABO antibodies | Gender | Number | Median (range) of | | | |
|--------------------|--------|--------|-------------------------------|-------------|-------------------------------|------------|
| | | | Antibody agglutination scores | | Antibody agglutination titers | |
| | | | IAT-CTT | IAT-HDM | IAT-CTT | IAT-HDM |
| Anti-A | Male | 51 | 62.0 (16-118) | 25.0 (3-63) | 256 (4-4096) | 8 (1-128) |
| | Female | 49 | 60.0 (16-108) | 21.0 (5-65) | 256 (8-2048) | 4 (1-128) |
| Anti-B | Male | 51 | 64.0 (3-112) | 23.0 (3-65) | 256 (1-2048) | 8 (1-128) |
| | Female | 49 | 60.0 (8-101) | 23.0 (3-97) | 128 (2-2048) | 8 (1-1024) |

IAT, indirect antiglobulin test; CTT, conventional tube test; HDM, hydrogel medium

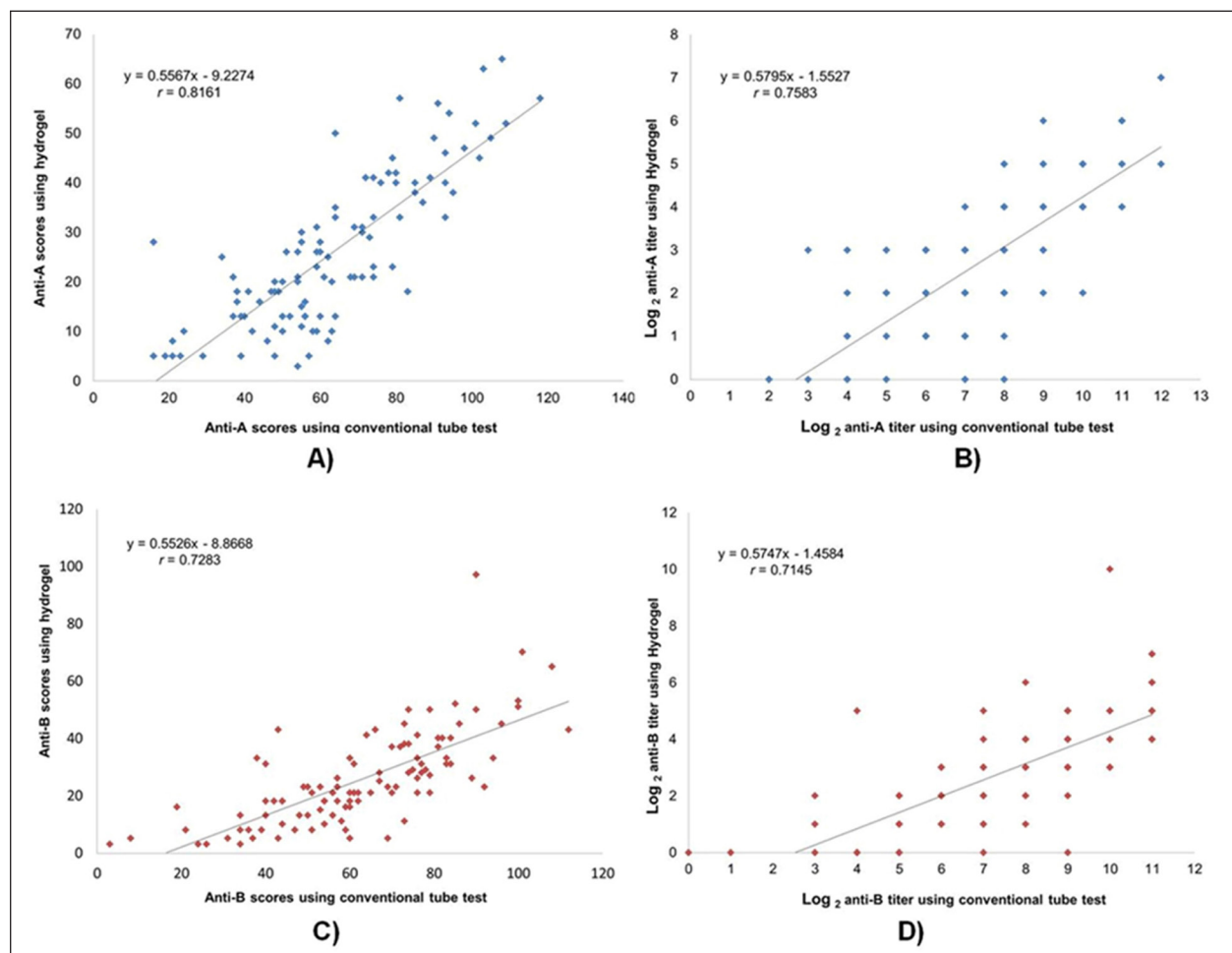


Figure 2 Correlation of IgG ABO antibody scores and titers between CTT and HDM in 100 group O Thai blood donors

donors determined by IAT using CTT and HDM was analyzed, as shown in Figure 2. Pearson correlation (r) was confirmed that 2 data were correlated in case of the r value was positive. The r value of IgG anti-A agglutination scores obtained by CTT versus HDM was 0.8161 ($p < 0.05$) with the regression equation of $y = 0.5567x - 9.2274$ (Figure 2A). Whereas, the

r value of IgG anti-A titers was 0.7583 ($p < 0.05$) with the regression equation of $y = 0.5795x - 1.5527$ (Figure 2B). Additionally, the r values of IgG anti-B scores and titers were 0.7283 ($p < 0.05$) with $y = 0.5526x - 8.8668$ (Figure 2C), and 0.7145 ($p < 0.05$) with $y = 0.5747x - 1.4584$ (Figure 2D), respectively.

Table 3 Reproducibility of the sum of IgG anti-A and anti-B agglutination scores determined by IAT using HDM

| | Anti-A scores | | | Anti-B scores | | |
|-------------|---------------|-------|-------|---------------|-------|-------|
| | No.1 | No.2 | No.3 | No.1 | No.2 | No.3 |
| Mean | 38.40 | 35.00 | 26.00 | 47.60 | 34.80 | 24.80 |
| SD | 0.89 | 1.41 | 0.00 | 0.89 | 1.00 | 1.10 |
| %CV | 2.33 | 4.04 | 0.00 | 1.88 | 3.10 | 4.40 |

SD, standard deviation; CV, coefficient of variation

Reproducibility test for IAT using HDM

To assess the reproducibility of IgG anti-A and anti-B titers by IAT using HDM, the agglutination titers in 3 serum samples were performed in quintuplicate. The mean, standard deviation (SD) and coefficient of variation (CV) values of the sum of agglutination scores were determined (Table 3). The CV values of three different serum samples were 2.33%, 4.04% and 0.00% for anti-A and 1.88%, 3.10%, and 4.40% for anti-B, respectively.

Discussion

According to the requirements of the Standards for Blood Banks and Transfusion Services, it is recommended that the blood bank have a policy concerning transfusion of components, which contain significant amounts of ABO incompatible antibodies.²³ Therefore, screening for donor anti-A and anti-B hemolysins, and high titers of IgM and IgG is suggested when using platelets containing ABO incompatible plasma.²⁴ Additionally, ABO antibody titration is important especially in cases of ABO-HDFN and ABO incompatible stem cell and solid organ transplantations.^{4,20} However, using different techniques for IgG ABO antibody titration will affect the test results.

In this study IgG anti-A and anti-B titers in 100 group O Thai blood donors were determined by IAT using CTT and HDM. There was no association between gender and age with IgG anti-A and anti-B titers similar to a previous study.²⁵ On the contrary, IgG titers of anti-A and anti-B in Japanese donors showed differences between sexes and increase in donor

age, especially in female blood donors.²⁶ Comparing the sum of IgG ABO antibody agglutination scores between using IAT-CTT and IAT-HDM, we found that they were significantly higher in CTT than HDM. However, hemagglutination titers of both IgG anti-A and anti-B showed good correlation between the two techniques. Hence, the use of IAT-HDM could be accomplished as an alternative test for IgG antibody detection. A recent study demonstrated that the sensitivity for the detection of anti-D by IAT-HDM was consistent with that of the IAT-CTT, but lower than that of the gel test. Moreover, for IAT-HDM, the residual amount of non specific unbound globulin in the pellet is 4 µg/mL.¹⁹

The advantages of using IAT-HDM are first, the quintuplicate agglutination scores showed no significant difference. Second, its use is simple and less time-consuming because a rapid single-step centrifugation is required for IAT. However, the cost required for HDM is still not available to be considered as a commercial kit. Even though, IAT-CTT is the standard method for IgG antibody detection; however, IAT-HDM might be applied for routine blood bank laboratories. Notably, to determine clinically significant titers, especially in patients receiving high-dose IVIG treatment, further studies in evaluation of appropriate anti-A and anti-B titers using IAT-HDM is suggested. In addition, for Rh and other potentially significant antibodies capable of causing HDFN, antibody titration using IAT-HDM should be standardized.

In conclusion, the IAT-HDM can be used for determination of IgG ABO antibody titers in order to

eliminate washing step. Moreover, it provides reliable results and reproducible testing but the antibody titers tested by IAT-HDM were less than IAT-CTT.

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การศึกษาเปรียบเทียบการตรวจหาความแรงของแอนติบอดีชนิด IgG ต่อหมู่โลหิตเอบีโอ ด้วยวิธีหลอดทดลองและใช้น้ำยา Hydrogel

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บทคัดย่อ

บทนำ แอนติบอดีของหมู่โลหิตเอบีโอเป็นแอนติบอดีที่มีความสำคัญทางเวชศาสตร์การบริการโลหิต การตรวจ ABO antibody titration ใช้ในการประเมินผลการรักษา โดยเฉพาะอย่างยิ่งกรณีปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตและปลูกถ่ายอวัยวะที่มีหมู่โลหิตเอบีโอเข้ากันไม่ได้ อีกทั้งยังใช้ประเมินการตอบสนองต่อการให้ส่วนประกอบของโลหิตที่มีหมู่โลหิตเอบีโอเข้ากันไม่ได้ **วัตถุประสงค์** เพื่อศึกษาการตรวจหาแอนติบอดีของ anti-A และ anti-B ชนิด IgG ในผู้บริจาคโลหิตคนไทยหมู่โอ ด้วยวิธี indirect antiglobulin test (IAT) โดยวิธีหลอดทดลอง และ ใช้น้ำยา hydrogel **วัสดุและวิธีการ** ใช้ตัวอย่างซีรัมของผู้บริจาคโลหิตหมู่โอจำนวน 100 ราย จากศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ทำการตรวจหาความแรงของ IgG anti-A และ anti-B โดยใช้การทดสอบ IAT ด้วยวิธีหลอดทดลอง และ ใช้น้ำยา hydrogel เพื่อลดขั้นตอนการล้างเซลล์ ทำการเปรียบเทียบทั้งผลรวมคะแนนและความแรงของปฏิกิริยาการจับกลุ่มที่ได้จาก 2 วิธี **ผลการศึกษา** จากตัวอย่างผู้บริจาคโลหิต 100 ราย ช่วงอายุตั้งแต่ 18 ปี ถึง 58 ปี เป็นชาย 51 ราย และหญิง 49 ราย (ชาย:หญิง = 1:1) จากการศึกษาพบว่า ความแรงและผลรวมคะแนนปฏิกิริยาการจับกลุ่มของ IgG anti-A และ anti-B ไม่มีความสัมพันธ์กับอายุและเพศ สำหรับการทดสอบหาความแรงของ anti-A และ anti-B ชนิด IgG ด้วยวิธีหลอดทดลองนั้นให้ผลคะแนนสูงกว่าการใช้น้ำยา hydrogel ($p < 0.001$) อย่างไรก็ตามเมื่อวิเคราะห์ความสัมพันธ์ของผลรวมคะแนนปฏิกิริยาการจับกลุ่มทั้ง IgG anti-A และ anti-B ระหว่าง 2 วิธี พบว่า มีความสัมพันธ์อยู่ในเกณฑ์ดี (anti-A, $r = 0.8161$ และ anti-B, $r = 0.7283$) นอกจากนี้ได้ประเมินความสามารถในการทำซ้ำของการตรวจหาผลรวมคะแนนปฏิกิริยาการจับกลุ่มทั้ง IgG anti-A และ anti-B ด้วยการใช้ น้ำยา hydrogel ในตัวอย่างซีรัม 3 ราย ที่ทำการทดสอบซ้ำ 5 ครั้ง โดยศึกษาค่าเฉลี่ย ค่าเบี่ยงเบนมาตรฐานและค่าสัมประสิทธิ์ความแปรปรวน (coefficient of variation, CV) พบว่า ค่า CV ของผลรวมคะแนนปฏิกิริยาการจับกลุ่มน้อยกว่าร้อยละ 5 **สรุป** การศึกษาครั้งนี้พบว่า การใช้น้ำยา hydrogel สำหรับการตรวจ IAT ช่วยลดขั้นตอนการล้างเซลล์ นอกจากนี้ผลการทดสอบที่ได้มีความน่าเชื่อถือและเมื่อทดสอบซ้ำก็ให้ผลที่แม่นยำ แต่ให้ค่าความแรงต่ำกว่าวิธีหลอดทดลอง

Keywords : ● ABO antibodies ● Hydrogel ● Group O ● Thai

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