ORIGINAL REPORT

# Comparative evaluation of the conventional tube test and column agglutination technology for ABO antibody titration in healthy individuals: a report from India

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Determination of accurate anti-A/-B titers is important for treatment selection in ABO-incompatible stem cell and solid-organ transplants. The standard method for ABO antibody titration is the conventional tube test (CTT). Dithiothreitol (DTT) is commonly used to inactivate the IgM antibody component. The aim of this study was to compare six different methods for ABO antibody titration and to observe the effectiveness of DTT on antibody estimation. A total of 90 healthy voluntary blood donors were enrolled in this study, including 30 each for blood groups A, B, and O. Antibody titrations were performed and tested using the CTT-immediate spin (IS), CTT-antihuman globulin (AHG) with and without DTT, column agglutination technology (CAT)-IS, and CAT-AHG with and without DTT methods. Bead-CAT was used, and the positive cutoff value was set to 1+ for each method to determine the endpoint of the titer. The median values of anti-A/-B titers by IS were found to be higher than those values by AHG in CTT and CAT among group B and A individuals, whereas no statistically significant differences were observed in values from group O individuals for IS and AHG anti-A/-B titers, estimated by each method. Although there was positive correlation between the anti-A/-B titer results obtained using the CTT and CAT in all blood groups, testing using AHG showed poor agreement with and without DTT pretreatment (kappa value of 0.11 and 0.20, respectively). Moderate agreement was observed between CTT-IS and CAT-IS (kappa value of 0.46). Median anti-A/-B AHG titers were reduced by the use of DTT in all blood group samples. Significant differences in the interpretability of anti-A/-B titers were observed among different methods. A uniform approach for selecting the method for ABO antibody titration is highly recommended, and DTT pretreatment of plasma to neutralize IgM activity should be considered to obtain precise values of IgG anti-A/-B titers. ***Immunohematology* 2021;37:25–32.**

**Key Words:** ABO antibody, antibody titration, column agglutination technology, conventional tube test, IgG, IgM, dithiothreitol

ABO blood group antigens are called histo-antigens because they are known to be expressed not only on red blood cells (RBCs) but also on almost all other organs in the human body.[1] ABO antibodies are naturally occurring, characterized as causing hemolytic transfusion reactions, hemolytic disease of the fetus and newborn, and antibody-mediated rejection of solid-organ transplants. In ABO-incompatible stem cell transplants, anti-A/-B titer levels correlate with the risk of immediate or delayed hemolysis, delaying the engrafting of RBCs.[2] Both immunoglobulin (Ig)G and IgM anti-A/-B show hemagglutination with RBCs at or below room temperature and can activate complement at 37°C.[3] Determining accurate anti-A/-B titers is beneficial for treatment selection, since patients with high anti-A/-B titer undergoing ABO-incompatible transplantation may be treated with anti-CD20 monoclonal antibody infusion, double-filtration plasmapheresis, or plasma exchange to reduce ABO antibody levels before the transplantation.[4] The most common and recommended method for ABO antibody titration is the conventional tube test (CTT). Testing by the CTT-immediate spin (IS) using normal-ionic-strength saline is commonly used for determination of IgM antibody, and testing by the CTT-antihuman globulin (AHG) by indirect antiglobulin test (IAT) is recommended for determination of IgG reactivity.[3] Titers are generally determined using a semiquantitative assay by serial double-fold dilution; the titer cutoff value is reciprocal to the highest sample dilution showing 1+ agglutination. Dithiothreitol (DTT) is used for the inactivation of IgM antibodies and, therefore, its routine use in immunohematology laboratories is recommended.[5] Although the column agglutination technology (CAT) has been described as superior in objectivity and reproducibility when compared with CTT because of the possible overestimation of the antibody titer when using CAT, it has, up to now, only been recommended for antibody identification and not for standard antibody level determination.[6,7] Cho et al.[8] compared the effectiveness of CAT and CTT for ABO antibody titration; they suggested that CAT-AHG could be used to standardize ABO antibody titration at different institutions. They measured only total ABO antibody, however, and did not distinguish

between IgM and IgG. On the other hand, a study conducted by Kang et al.[9] investigated ABO antibody titer distribution of normal individuals using CTT-AHG with and without DTT to distinguish IgM and IgG reactivity. They performed all tests with CTT only, however. There are very few publications available in support of whether DTT can be used in CAT or whether DTT treatment of serum (or plasma) leads to false-positive reactions by CAT.[10] As cases of ABO-incompatible transplants are gradually increasing in resource-constrained developing countries across the globe, it has become extremely important for immunohematology laboratories to validate an accurate method to estimate precise IgM and IgG levels of reactivity in ABO titers in patients as well as in potential healthy organ or stem cell donors.

The aim of this study was to compare ABO titration results using six different methods available in our immunohematology laboratory: CTT-IS, CTT-AHG with DTT, CTT-AHG without DTT, CAT-IS, CAT-AHG with DTT, and CAT-AHG without DTT. Additionally, the effectiveness of using DTT in ABO titration at the AHG phase was evaluated during this study.

## Material and Methods

This observational study was conducted in the department of transfusion medicine at a tertiary care oncology center in eastern India. The study was conducted in 2018–2019 on healthy voluntary blood donors. The selection of blood donors was according to the guidelines stated in the Drug and Cosmetic Act 1940[11] and the Standards for Blood Banks and Blood Transfusion Services.[12] A total of 90 healthy individuals between the ages of 18 and 65 years were enrolled in this study. Written informed consent was obtained from each person before blood donation. As per the institutional policy, anti-A/-B titration is routinely performed on samples from blood donor units at our blood center; the intent was to avoid hemolytic reactions caused by transfusion of non–group-specific platelet concentrates. Therefore, separate institutional review board approval was not obtained for this study. Thirty individuals, each of blood groups A, B, and O, were enrolled. Table 1 shows the characteristics of the enrolled individuals. Sample size was calculated on the basis of prevalence of the ABO blood groups in this part of the world as per previously published literature.[13]

Samples were taken from the diversion pouch of the blood collection bag for routine donor testing (e.g., ABO and D testing and screening for irregular RBC antibodies) as well as for this study. Samples for this study were collected into EDTA vials (BD Vacutainer; BD, Franklin Lakes, NJ) and stored at 2–6°C. All samples were tested for ABO/D and screened for irregular RBC antibodies on the fully automated immunohematology analyzer (VISION Analyzer for BioVue; Ortho Clinical Diagnostics, Pencoed, UK). All samples were negative for irregular RBC antibodies and were nonreactive for transfusion-transmitted diseases.

**Table 1.** Characteristics of the enrolled individuals

| Blood group | Samples, *n* | Gender, male/female | Age in years, mean ± standard deviation |
|---|---|---|---|
| A | 30 | 22/8 | 28.18 ± 6.63 |
| B | 30 | 24/6 | 29.90 ± 6.74 |
| O | 30 | 25/5 | 31.73 ± 9.54 |
| Total | 90 | 71/19 | 30.61 ± 8.48 |

The EDTA samples were centrifuged for 10 minutes at 1400*g* to obtain the plasma for antibody titer determination by six methods: CTT-IS, CTT-AHG with DTT, CTT-AHG without DTT, CAT-IS, CAT-AHG with DTT, and CAT-AHG without DTT. Testing was performed within 24 hours of whole blood collection. The serial doubling dilutions were prepared, and the titrations were performed by two technologists. The results were interpreted by one physician and data collected by another to avoid interobserver variability. Final data analysis was performed independently by a statistician.

### Antibody Titration Methods

#### CTT-IS

Anti-A/-B titration using CTT-IS was performed according to the method described in the AABB Technical Manual[3] and as per departmental standard operating procedure. Normal-ionic-strength saline was used as diluent. For each donor sample, 100 µL of each serial dilution of the donor's plasma was pipetted into 10–12 prelabeled clean test tubes. One drop each of reagent RBCs ($A_1$ and B Affirmagen; Ortho Clinical Diagnostics) was added to the appropriate tube. After incubation at ambient (22–25°C) room temperature, tubes were centrifuged at 1000*g* for 25 seconds. Agglutination was read macroscopically, and titer was determined as the reciprocal of the highest dilution showing 1+ agglutination.

#### CTT-AHG With and Without DTT

The IAT was used to determine the anti-A/-B AHG phase titer. The prelabeled tubes containing serially diluted donor plasma and reagent RBCs were incubated at 37°C for 45 minutes. After the incubation, the tubes were washed three

times using normal saline. Two drops of polyspecific AHG (Tulip Diagnostics, Goa, India) were added after discarding the last supernatant, and the tubes were finally centrifuged at 1000*g* for 15 seconds. Agglutination reactivity and titer endpoint were interpreted in a manner similar to that used for CTT-IS.

To determine the IgG antibody titer, the plasma samples were evaluated by IAT after pretreatment of plasma with an equal volume of 0.01 M DTT at 37°C for 60 minutes (0.01 M DTT was prepared by dissolving 0.154 g DTT [Himedia Laboratory, Mumbai, India] in 100 mL of pH 7.3 phosphate-buffered saline). Testing followed the same procedure as that used for the CTT-AHG without DTT, and results were interpreted after adjusting the dilution factor due to DTT pretreatment of plasma.

### CAT-IS

Anti-A/-B titration using the CAT-IS was performed in reverse diluent cards (MTS Reverse Group Diluent Card; Ortho Clinical Diagnostics) that were labeled separately for anti-A and anti-B. The CAT used bead-column agglutination. Diluted serum (40 µL) and 10 µL of $A_1$ and B reagent RBCs were dispensed into separate microcolumns and centrifuged for 5 minutes in a dedicated centrifuge for CAT (MTS, BioVue System; Ortho Clinical Diagnostics). The cutoff value for the agglutination grading using CAT-IS was set to 1+, similar to that used for CTT, and interpretation was determined in the same manner.

### CAT-AHG With and Without DTT

Anti-A/-B titration using CAT was performed in polyspecific AHG cards (MTS AHG Card; Ortho Clinical Diagnostics) that were labeled separately for anti-A and anti-B. Diluted serum (40 µL), 10 µL of $A_1$ and B reagent RBCs, and 50 µL of low-ionic-strength solution were dispensed into separate microcolumns, followed by 15-minute incubation at 37°C and 5-minute centrifugation in a dedicated centrifuge for CAT (MTS, BioVue System; Ortho Clinical Diagnostics). The titer endpoint was the reciprocal of the highest dilution yielding 1+ agglutination.

Plasma samples were also evaluated by CAT using anti-IgG cards (MTS IgG Card; Ortho Clinical Diagnostics) after pretreatment of plasma with DTT. (Pre-study validation of DTT on the CAT was performed by using plasma containing known amounts of IgG and IgM antibodies as positive control.) An additional control was run in parallel using plasma containing IgG only along with the reagent RBCs (Affirmagen $A_1$ and B RBCs; Ortho Clinical Diagnostics) in the presence of DTT to exclude any DTT interference with the test method. Plasma pretreatment with DTT and testing of the serial dilutions were performed as described under CTT-AHG with DTT. The results were interpreted in microcolumns after adjusting the dilution factor due to DTT pretreatment of plasma.

### Statistical Analysis

The distributions of antibody titers according to age-group and gender were evaluated and compared by analysis of variance and the two-sample *t* test, respectively. The consistency between the antibody titer measured by the CTT and CAT was evaluated using kappa analysis. Furthermore, the correlation between the CTT and CAT was evaluated by calculating the Pearson correlation coefficient through linear regression analysis. Data were primarily collected and analyzed using a statistics software (Excel; Microsoft, Redmond, WA). Further statistical calculation was performed (R software, version 3.4.2; The R Project for Statistical Computing, Vienna, Austria), and $p$ values <0.05 were considered statistically significant.

## Results

The distribution of anti-A/-B titers according to age-group is shown in Table 2. There were no statistically significant differences among age-groups. Table 3 shows ABO titer distribution according to gender; no statistical differences were observed between male and female individuals. The distribution of ABO titers according to titration methods is shown in Table 4. The median values of anti-A/-B titers by IS were found higher than those found by AHG in both CTT and CAT among group B and A individuals ($p < 0.05$ for all methods), whereas no statistically significant differences were observed in group O individuals between IS and AHG anti-A/-B titers, estimated by each method. A moderate agreement was observed between CTT-IS and CAT-IS (kappa value of 0.46), as shown in Table 5. Moreover, Table 5 shows poor agreement between the CTT-AHG and CAT-AHG titers with and without DTT pretreatment (kappa value of 0.11 and 0.20, respectively). A positive correlation was observed between the anti-A/-B titer results obtained using the CTT and CAT ($p < 0.01$ for each method) among all individuals (Table 6). Median anti-A/-B AHG titers were reduced after DTT treatment in samples from all blood groups, regardless of method, as shown in Table 4. Figure 1 compares the differences

**Table 2.** Distribution of ABO antibody titers according to age–range groups

| ABO antibody | Samples, *n* | Age range, years | Antibody median titer (interquartile range) for each method | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | CAT-IS | CAT-AHG with DTT | CAT-AHG without DTT | CTT-IS | CTT-AHG with DTT | CTT-AHG without DTT |
| Anti-A in blood group B | 16 | 18–29 | 32 (16–32) | 2 (0–4) | 16 (8–64) | 16 (8–16) | 0 (0) | 4 (4–16) |
| | 11 | 30–39 | 32 (16–96) | 4 (2–10) | 32 (12–96) | 16 (12–32) | 0 (0–1) | 8 (4–16) |
| | 1 | 40–49 | 32 | 0 | 8 | 16 | 0 | 2 |
| | 2* | 50–65 | 32 (32) | 2 (1–2) | 18 (4–32) | 12 (8–16) | 0 (0) | 6 (4–8) |
| Total | 30 | | | | | | | |
| Anti-B in blood group A | 17 | 18–29 | 32 (16–64) | 4 (1–8) | 64 (8–64) | 32 (8–64) | 1 (0–2) | 16 (7–32) |
| | 8 | 30–39 | 16 (4–28) | 4 (1–8) | 16 (7–32) | 8 (4–40) | 0 (0–1) | 30 (4–28) |
| | 3* | 40–49 | 64 (16–64) | 2 (2) | 32 (16–32) | 64 (16–64) | 0 (0–8) | 32 (0–32) |
| | 2* | 50–65 | 16 (16) | 2 (0–4) | 12 (8–16) | 10 (4–16) | 0.5 (0–1) | 8 (8) |
| Total | 30 | | | | | | | |
| Anti-A in blood group O | 15 | 18–29 | 256 (96–256) | 128 (48–256) | 256 (128–384) | 64 (32–64) | 8 (1–16) | 32 (16–64) |
| | 10 | 30–39 | 192 (80–256) | 32 (32–64) | 256 (128–448) | 48 (20–64) | 4 (1–8) | 48 (20–112) |
| | 3* | 40–49 | 256 (32–256) | 64 (32–64) | 128 (64–128) | 16 (16–32) | 8 (2–16) | 16 (16–32) |
| | 2* | 50–65 | 80 (32–128) | 96 (64–128) | 136 (16–256) | 12 (8–16) | 24 (16–32) | 16 (0–32) |
| Total | 30 | | | | | | | |
| Anti-B in blood group O | 15 | 18–29 | 128 (128–512) | 128 (48–192) | 128 (128–512) | 64 (48–128) | 4 (2–12) | 32 (16–48) |
| | 10 | 30–39 | 288 (37–512) | 96 (40–128) | 512 (160–512) | 32 (32–64) | 6 (1–16) | 32 (20–64) |
| | 3* | 40–49 | 256 (16–512) | 64 (2–64) | 128 (128–256) | 32 (8–32) | 4 (0–16) | 32 (16–32) |
| | 2* | 50–65 | 128 (128) | 192 (128–256) | 144 (32–256) | 24 (16–32) | 48 (32–64) | 66 (4–128) |
| Total | 30 | | | | | | | |

*Upper and lower ranges of titers reported instead of interquartile range.
CAT = column agglutination technology; IS = immediate spin; AHG = antihuman globulin; DTT = dithiothreitol; CTT = conventional tube test.

**Table 3.** Distribution of ABO antibody titers according to gender

| ABO antibody | Samples, *n* | Gender | Antibody median titer (interquartile range) for each method | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | CAT-IS | CAT-AHG with DTT | CAT-AHG without DTT | CTT-IS | CTT-AHG with DTT | CTT-AHG without DTT |
| Anti-A in blood group B | 24 | Male | 32 (16–64) | 2 (0–4) | 16 (8–64) | 16 (8–16) | 0 (0–1) | 8 (4–16) |
| | 6 | Female | 32 (32) | 2 (2) | 24 (16–32) | 16 (16–28) | 0 (0) | 4 (4) |
| Total | 30 | | | | | | | |
| Anti-B in blood group A | 22 | Male | 16 (16–64) | 4 (1–8) | 16 (10–64) | 16 (8–32) | 1 (0–2) | 12 (5–28) |
| | 8 | Female | 48 (16–80) | 5 (2–8) | 48 (8–80) | 64 (8–128) | 1 (0–2) | 32 (7–40) |
| Total | 30 | | | | | | | |
| Anti-A in blood group O | 25 | Male | 256 (64–256) | 64 (32–128) | 256 (128–256) | 32 (16–64) | 4 (1–8) | 32 (16–64) |
| | 5 | Female | 256 (128–256) | 256 (256) | 128 (64–512) | 64 (32–64) | 16 (16–32) | 64 (32–64) |
| Total | 30 | | | | | | | |
| Anti-B in blood group O | 25 | Male | 256 (64–512) | 128 (32–128) | 256 (128–512) | 32 (32–64) | 4 (1–16) | 32 (16–64) |
| | 5 | Female | 128 (128) | 128 (64–128) | 64 (32–128) | 64 (32–64) | 16 (4–32) | 16 (16–32) |
| Total | 30 | | | | | | | |

CAT = column agglutination technology; IS = immediate spin; AHG = antihuman globulin; DTT = dithiothreitol; CTT = conventional tube test.

**Table 4.** Distribution of ABO antibody titers according to titration method

| ABO antibody | Samples, *n* | Antibody median titer (interquartile range) for each method | | | | | |
|---|---|---|---|---|---|---|---|
| | | CAT-IS | CAT-AHG with DTT | CAT-AHG without DTT | CTT-IS | CTT-AHG with DTT | CTT-AHG without DTT |
| Anti-A in blood group B | 30 | 32 (16–32) | 2 (0–4) | 16 (8–64) | 16 (8–16) | 0 (0) | 6 (4–16) |
| Anti-B in blood group A | 30 | 24 (16–64) | 4 (1–8) | 24 (8–64) | 24 (8–64) | 1 (0–2) | 16 (5–32) |
| Anti-A in blood group O | 30 | 256 (64–256) | 64 (32–128) | 192 (128–256) | 32 (16–64) | 8 (1–16) | 32 (16–64) |
| Anti-B in blood group O | 30 | 128 (64–512) | 128 (40–128) | 256 (128–512) | 48 (32–64) | 6 (2–16) | 32 (16–64) |

CAT = column agglutination technology; IS = immediate spin; AHG = antihuman globulin; DTT = dithiothreitol; CTT = conventional tube test.

**Table 5.** Consistency between ABO antibody titer results obtained using CTT and CAT with and without DTT treatment

| Method | Concordance rate (%) | Cohen's kappa |
|---|---|---|
| CTT-IS vs. CAT-IS | 0.72 | 0.46 |
| CTT-AHG vs. CAT-AHG (with DTT) | 0.48 | 0.11 |
| CTT-AHG vs. CAT-AHG (without DTT) | 0.52 | 0.20 |

CTT = conventional tube test; CAT = column agglutination technology; DTT = dithiothreitol; IS = immediate spin; AHG = antihuman globulin.

**Table 6.** Correlation between ABO antibody titer results obtained using CTT and CAT methods among all participants

| Method | Pearson correlation coefficient | *p* |
|---|---|---|
| CTT-IS vs. CAT-IS | 0.70 | <0.01 |
| CTT-AHG vs. CAT-AHG (with DTT) | 0.98 | <0.01 |
| CTT-AHG vs. CAT-AHG (without DTT) | 0.92 | <0.01 |

CTT = conventional tube test; CAT = column agglutination technology; DTT = dithiothreitol; IS = immediate spin; AHG = antihuman globulin.

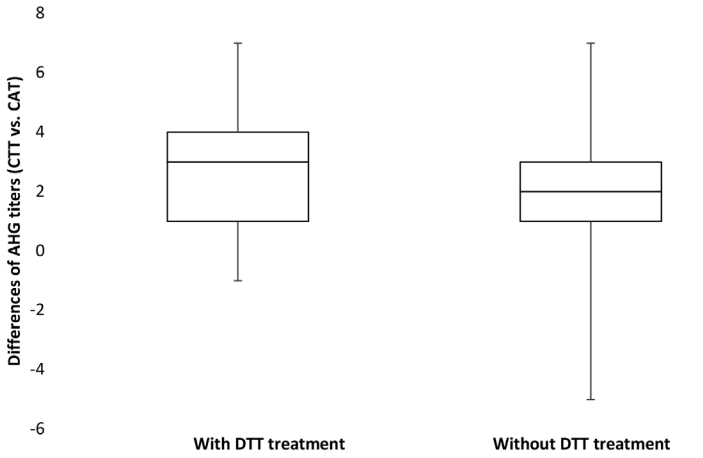


**Fig. 1** Differences of ABO-antibody antihuman globulin (AHG) titers between conventional tube test (CTT) and column agglutination technology (CAT) with and without dithiothreitol (DTT) treatment. The box plot shows AHG antibody titer differences between samples treated or not treated with DTT. In the box plot, the central rectangle spans the first quartile to the third quartile. A segment inside the rectangle shows the median, and whiskers above and below the box show the locations of the minimum and maximum values. The figure reveals that the median difference is greater in the DTT-treated group than in the group without DTT treatment (CTT vs. CAT).

in the antibody titer results between CTT-AHG and CAT-AHG with and without DTT treatment.

## Discussion

Significant differences in the interpretability of anti-A/-B titers were observed among different testing methods in this study. Until now, no standard serologic method for ABO antibody titration has been established in most of the immunohematology laboratories across the Asia-Pacific region.[14,15] The ABO antibody detection capability for each detection method varies significantly; therefore, knowing the differences between titer distributions exhibited by each method and each institution could be important. The advantage of CAT is that it may reduce the interobserver and interinstitutional variation, and analysis time could be shortened, as demonstrated by Shirey et al.[16] In this study, we performed ABO titrations by six different methods. The median values of ABO antibodies by CTT and CAT in our study were similar to those of Park et al.,[17] but the interquartile ranges were narrower than theirs, which could be due to differences in sample size between the two studies.

We found that the median values of anti-A/-B titers by IS were higher than those by AHG both in CTT and CAT in group B and A individuals, whereas almost similar IS and AHG titer results were observed in group O individuals. This finding is similar to that from the study conducted by Kang et al.,[18] where flow cytometry was used as a gold-standard test method. This observation might be because IgM anti-A/-B is the predominant isotype found in group B and group A

individuals, whereas IgG ABO is present in a significant amount in group O individuals.[3] In addition to that, group O individuals also produce anti-A,B.[3] The fact that anti-A,B does not contain separable anti-A and anti-B components might cause some interference in the anti-A/-B titer results in group O individuals.

Moderate agreement was observed between CTT-IS and CAT-IS, but agreement was poor between CTT-AHG and CAT-AHG titers with and without DTT pretreatment in this study; this finding is quite similar to results published by Nayak et al.[19] The high value of anti-A/-B titers observed in CAT in our study could be due to microcolumns using glass beads as the column component or the presence of 3 percent polyethylene glycol in reverse diluent cassettes. The study by Tanabe[20] also reported a similar observation when various CAT methods were compared; the study suggested that the bead-column agglutination method shows high maximum titer compared with other gel-column agglutination methods because the serum-to-cell ratio is slightly higher in CAT using glass beads. Because serum-to-cell ratios suggested by manufacturers' instructions are different depending on the column component, it is recommended to distinguish between the two CAT methods when comparing target levels of ABO antibody titer. A positive correlation that was observed in this study between the anti-A/-B titer results obtained using the CTT and CAT is in agreement with other published literatures.[10,19] Antibody titration with DTT-treated plasma can reduce the impact of IgM anti-A/-B on the measurement of IgG ABO by breaking the disulfide bonds that hold the structure of the IgM pentameric molecule. Moreover, the polyspecific AHG used in the CAT could cross-react with IgA and IgM, as well as with the complement components, which could potentially cause false elevation in AHG titer measurements by the IAT method due to carry-over of reactivity of IgM anti-A/-B at 37°C.

In our study, we compared the antibody titer obtained using DTT-treated or untreated plasma samples and found that the median value of anti-A/-B AHG titers was reduced by DTT treatment in samples from all blood groups tested in each method. The differences of AHG titer results were compared between CAT and CTT, and the median difference was found slightly higher in DTT-treated samples than in DTT-untreated samples. No evidence of falsely elevated titers due to DTT treatment of the plasma samples was observed using CAT, excluding the possibilities of positive bias by DTT pretreatment during CAT. Our observations strongly suggest that DTT treatment is needed to measure the exact IgG anti-A/-B titers, and, therefore, DTT-treated plasma should be tested with anti-IgG in the AHG phase to obtain the precise IgG titers of ABO antibodies.

The wide variability of the titer results among laboratories as well as among the different methods that are commonly being used for ABO antibody titration is a matter of great concern, especially for ABO-incompatible transplantations.[21,22] If the results for the antibody titer are found falsely elevated by a new method, then treatment such as plasma exchange that is not necessary for a specific patient might be considered inadvertently, which could ultimately increase the cost of the treatment. On the contrary, if the titration results are found lower than those with the conventional method, patients may be deprived of receiving a potential treatment. As most transplant physicians remain focused only on the target titer without taking into account the differences that may arise in these values due to the choice of the method used, we recommend that they must be cautious in interpreting the results of ABO titer, and laboratories should provide appropriate information on the method of testing to facilitate accurate interpretation of results.

There are some limitations in our study, including a relatively small sample population. Comparison with flow cytometry, which is a gold-standard test for titer estimation, could not be carried out because of the higher cost of the method; this lack of testing could be considered another limitation. Moreover, the variation between CAT and CTT could be reduced if we use titer endpoints of 1+ in CAT and w+ in CTT as suggested by AuBuchon et al.[23] In our study, we compared CTT with CAT, which uses a glass-bead column, but there are other CAT formulations available that use gel columns, which may give different results.

To reduce the variation of titration results between and within different methods, an ABO titration external quality assurance (EQA) program is urgently needed for most of the laboratories in the Asia-Pacific region. Development of a standard method and participation in an EQA program should, over time, reduce variation and enable transferrable results across testing centers, which will assist in consistent clinical interpretation.[14] New advancements like clinical application of kodecytes may be considered in contrast to natural cells for ABO titrations. The level of ABO antigen on kodecytes can be precisely controlled, and it has the potential to be standardized globally, thus resulting in more accurate clinical interpretation of ABO titer results.[24] In addition, the application of kodecytes for ABO titration in EQA programs needs to be explored in the future.

Recently, it was demonstrated that A and B subtype antigens are expressed differentially between erythrocytes and organs; for example, only subtype II A/B antigens are expressed on cardiac endothelium; thus, only antibodies against A or B subtype II antigens should be considered donor specific in the setting of ABO-incompatible cardiac transplantation.[25] Erythrocyte agglutination methods (CTT/CAT) provide limited information on antibody isotype determination, since they do not differentiate ABO subtypes. Therefore, new methods are developing in the form of ABH-glycan microarray or Luminex bead based–solid-phase, which will be used in the near future for accurate determination of donor-specific ABO subtypes in the setting of ABO-incompatible organ transplantation.[26,27]

Finally, we conclude that a uniform approach of selecting the method for ABO titration is highly necessary, and it is strongly recommended that caution be exercised in interpreting the ABO titer, taking the method used into consideration, particularly during selection of treatment protocol for ABO antibody neutralization.

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