Comparison of titer results obtained using immediate spin one-dilution techniques to a reference method

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BACKGROUND: Many transfusion services determine the titer of potentially incompatible plasma-containing products by performing a one-dilution titer at their selected titer threshold. This study compared the results of immediate spin (IS) one-dilution titers determined by three methods with a reference standard method.

METHODS: Plasma-containing products from group A and O donors were titered using the participant's routine IS one-dilution titer method. No time or temperature incubations were performed, and antihuman globulin reagent was not used. The samples were then tested using a reference method, which was a saline tube test with a 1-hour room temperature incubation; antihuman globulin was not used in the reference method. The results of the one-dilution titer were then compared to that obtained in the reference method.

RESULTS: Nine centers participated in this study. There were 698 antibodies from 374 units tested by the manual IS tube one-dilution titer method; sensitivity was 0.88 (95% confidence interval [CI], 0.83–0.92), and specificity was 1.00 (95% CI, 0.98–1.00). There were 412 antibodies from 206 units tested by the manual and automated IS buffered gel card one-dilution titer method; sensitivity was 0.95 (95% CI, 0.91–0.98), and specificity was 0.87 (95% CI, 0.81–0.91). There were 98 antibodies from 49 units tested by an automated microplate IS one-dilution titer method; sensitivity was 0.76 (95% CI, 0.71–0.93), and specificity was 0.96 (95% CI, 0.92–0.99). All three methods had an accuracy rate of 90% or greater.

CONCLUSION: The manual and automated one-dilution titer methods are suitable for screening plasma-containing units, although more evaluation of the automated microplate method might be required.

Due to shortages of universally compatible group AB plasma-containing blood products, some transfusion services have implemented programs for their trauma patients or those with massive bleeding whereby potentially incompatible plasma-containing products are transfused. For example, 58% of the surveyed Level I trauma centers in the United States in 2015 reported using group A plasma units in the resuscitation

ABBREVIATIONS: AHG = antihuman globulin; IS = immediate spin; LTOWB = low-titer group O whole blood; RT = room temperature; SDP = single-donor platelets; WB = whole blood.

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of trauma patients of unknown ABO group.[1] The use of low-titer group O whole blood (LTOWB) in this patient population is also becoming more widely adopted in the United States.[2] Similar policies of issuing potentially incompatible plasma-containing products to stable patients, especially when the products are scarce, such as HLA-matched single-donor platelet (SDP) units, are also in place at many institutions.[3] The AABB *Standards* permit the transfusion of incompatible plasma-containing products, including for the first time in the 31st edition of the *Standards* the use of incompatible plasma in LTOWB, so long as the transfusion service has a policy that directs that practice.[4] The AABB *Standards* do not provide specific guidance on what constitutes a high-titer antibody threshold or the method by which the titer of the antibodies in the potentially incompatible plasma should be determined. Thus, each transfusion service must decide on these details based on their own tolerance of hemolytic risk versus the exclusion of large numbers of donor units; transfusion services with a low tolerance for hemolytic risk favor low titers of antibodies in products that contain potentially incompatible plasma; however, this approach might exclude large numbers of donors if the titer threshold is set very low. In fact, a transfusion service policy of not titering the potentially incompatible antibodies would indeed be compatible with these standards; in the aforementioned plasma survey, of the US Level I trauma centers that issue group A plasma to trauma patients of unknown ABO group, 27 of 34 (79%) do not titer the potentially incompatible anti-B in the group A units.[1]

To mitigate the risk of hemolysis from the transfusion of potentially ABO-incompatible plasma-containing products, some centers issue these products only when the titer of the potentially incompatible antibodies are below a certain threshold.[5] For example, all of the surveyed US centers that provide LTOWB for trauma patients use units with antibodies that are below a certain threshold.[2] As it is advantageous for a transfusion service or blood center to screen numerous potentially incompatible plasma-containing products for high-titer antibodies at once, a rapid method for detecting high-titer antibodies is desirable. One solution is to make a single dilution of the plasma at the titer threshold and then test the diluted plasma against reagent A_1 and/or B RBCs. For example, a center that uses a titer threshold of less than 50 for its potentially incompatible plasma-containing products would mix 1 part of the donor plasma with 49 parts of diluent, thereby creating a 1:50 dilution, and then test this mixture against the appropriate reagent RBC(s). To expedite the testing, a one-dilution titer is often performed at immediate spin (IS) without an incubation of the diluted plasma and the reagent RBCs. Following centrifugation, RBC agglutination would indicate that the titer of the antibody in that plasma was at least 50; therefore, that plasma-containing product would not be issued in a potentially ABO-incompatible manner. This so-called IS one-dilution titer method is rapid and suitable for manual and automated testing.[6] However, it remains unclear how well the results of an IS one-dilution titer correlate to the results obtained in a titer method that includes an extended incubation period before centrifugation. The objective of this study was to compare the results of IS one-dilution titer tests performed by a variety of manual and automated methods to a standardized saline titer method featuring a 1-hour room temperature (RT) incubation.

METHODS

Participation was solicited from centers that were members of an international transfusion medicine research group. All institutions obtained the necessary research ethics review board approval to participate in this study. Each participating institution was asked to identify group A or O plasma-containing products, such as whole blood (WB), SDP, or plasma, that tested either positive or negative in their routine IS one-dilution titer method, and test them with the reference titer method (described below) to determine the performance characteristics of the IS one-dilution titer method. None of the SDP units were suspended in additive solution. Plasma-derived factor concentrates and intravenous immunoglobulin were not tested.

Seven institutions routinely used the manual tube or manual buffered gel card methods to perform the manual IS one-dilution titer test. One institution performed the IS one-dilution titer test on an automated buffered gel card instrument (Wadiana, Grifols). The IS one-dilution titer test performed on this instrument was a modification of the standardized IgM titer screen assay. The 1:64 dilution using solvent (DG Sol, Grifols) was performed by the instrument after it aspirated plasma from the unit undergoing testing and pipetted the solution into the gel card (DG Gel Neutral, Grifols). The instrument then added 50 μL of A_1 or B reagent RBC cells (Grifols). The reaction was read and interpreted by the instrument immediately after the centrifugation and standardized agitation steps. In both the manual tube and manual buffered gel card methods, and in the automated buffered gel card method, the following enhancements were not utilized: time or temperature incubation before centrifugation, use of potentiators such as low-ionic-strength saline or polyethylene glycol, or antihuman globulin (AHG) reagent. The results obtained using the manual and automated buffered gel card methods were combined and are reported together.

One institution performed the IS one-dilution titer test on an automated microplate instrument (NEO Iris, Immucor). The IS one-dilution titer test performed on this instrument was a modification of the standardized IgM titer screen assay. The 1:50 dilution using phosphate-buffered saline was performed by the instrument after it aspirated plasma from the unit undergoing testing. The instrument then added 15 μL of A_1 or B reagent RBC cells. The reaction

was read and interpreted by the instrument immediately after the centrifugation and standardized agitation steps. No potentiators or AHG reagent were used.

The results of the manual and automated IS one-dilution titer tests were compared to the results of a reference titer method that included a 1-hour RT incubation. The reference titer method involved preparing a dilution of the plasma-containing product at the institution's titer threshold in tube, and also at two dilutions below and above the titer threshold based on the standard doubling dilution strategy. For example, an institution that uses a titer threshold of 50 would have prepared a dilution of 1:50, as well as 1:16, 1:32, 1:64, and 1:128 for a total of five separate dilutions. The purpose of these four additional dilutions was to be able to estimate the magnitude of the error should the results of the IS one-dilution titer not coincide with that of the reference titer method. These five plasma dilutions were incubated for an hour at RT with the institution's routine A_1 and/or B reagent RBCs. No enhancement solutions, such as polyethylene glycol or low-ionic-strength saline, were added when performing the reference titer method. AHG was also not utilized in this reference titer method. At the end of the hour, the mixtures were centrifuged and inspected for agglutination; the same agglutination criteria for positivity that was used in each institution's routine IS one-dilution titer was employed in the reference method. Of the centers that performed the IS 1-dilution tube titer method, the positive titer endpoint was any macroscopic agglutination (i.e., macroscopic weak or +). At the centers that performed the IS one-dilution gel titer method, the positive titer endpoint was either weak or 1 ±.

The IS one-dilution titer results for all of the plasma-containing products at all titer thresholds were stratified by the method used to perform the IS one-dilution titer and compared to the corresponding results in the reference method. Descriptive statistics were calculated using computer software (Excel, Microsoft Corporation). In case of a discrepancy between the results of the IS one-dilution titer and the reference titer method, the latter was considered correct. The performance characteristics, including positive predictive value, negative predictive value, sensitivity, specificity, and accuracy, of each titer method were calculated using open access online calculators (http://vassarstats.net and https://www.medcalc.org/calc/diagnostic_test.php).

RESULTS

There were nine institutions that participated: four from the Czech Republic, four from the United States, and one from Brazil. The total number of samples tested, stratified by the nature of the plasma-containing products, and the anti-A and anti-B titer thresholds are shown in Table 1.

IS one-dilution titer performed by manual tube

In total, 698 antibodies from 374 group O WB and SDP units and group A and O plasma units were tested by both the institution's routine IS one-dilution tube titer method and by the reference tube titer method that included a 1-hour RT incubation. The IS one-dilution titer threshold for all of these group A and O units was 50 (Table 1). The performance characteristics of the IS one-dilution tube method are shown in Table 2. Overall, this method had an accuracy of 0.95 (95% CI, 0.93–0.97) (Table 2). Of the 698 antibodies tested by this method, there were 2 (0.3%) false-positive results and 32 (4.6%) false-negative results detected (Table 2). In the two false-positive results, the two lowest dilutions were positive in the reference method, but the threshold titer dilution and the two higher dilutions were all negative (Table 3). In the majority of the false-negative results (24 of 32; 75%), agglutination was detected in the reference titer method at the threshold titer but not at any dilutions above it.

IS one-dilution titer performed by manual and automated buffered gel card

In total, 412 antibodies from a total of 206 group O WB, SDP, and plasma units were tested by the institution's routine IS one-dilution buffered gel card titer method and also by the reference tube titer method that included a 1-hour RT incubation. Of these 206 units, 22 (10.7%) were tested using the automated instrument, and the remaining

TABLE 1. The number of plasma-containing blood products tested in this study stratified by the nature of the product and the anti-A and anti-B titer threshold

Blood products and titer thresholds		Manual tube	Manual and automated buffered gel card	Automated microplate
Number of plasma-containing blood products tested		374	206	49
Number of antibodies tested		698	412	98
Type of blood product tested	Group O WB	289 (77.3)	168 (81.6)	49 (100)
	Group O SDP	28 (7.5)	31 (15)	0 (0)
	Group O plasma	7 (1.9)	7 (3.4)	0 (0)
	Group A plasma	50 (13.4)	0 (0)	0 (0)
Titer threshold	1:50	374 (100)	184 (89.3)	49 (100)
	1:64	0 (0)	22 (10.7)	0 (0)

Data are presented as number (%).
SDP = single donor platelets; WB = whole blood.

TABLE 2. Performance characteristics of the different IS one-dilution titer methods evaluated in this study

	Manual tube	Manual and automated buffered gel card	Automated microplate
Number of false-positive results (%)	2 (0.3)	31 (7.5)	3 (3.1)
Number of false-negative results (%)	32 (4.6)	9 (2.2)	7 (7.1)
Number of true-positive results (%)	236 (33.8)	173 (42)	22 (22.4)
Number of true-negative results (%)	428 (61.3)	199 (48.3)	66 (67.3)
Sensitivity (95% CI)	0.88 (0.83–0.92)	0.95 (0.91–0.98)	0.76 (0.71–0.93)
Specificity (95% CI)	1.00 (0.98–1.00)	0.87 (0.81–0.91)	0.96 (0.92–0.99)
Positive predictive value (95% CI)	0.99 (0.97–0.99)	0.85 (0.79–0.89)	0.88 (0.80–0.98)
Negative predictive value (95% CI)	0.93 (0.90–0.95)	0.96 (0.92–0.98)	0.90 (0.88–0.97)
Accuracy (95% CI)	0.95 (0.93–0.97)	0.90 (0.87–0.93)	0.90 (0.82–0.95)

Data are presented as number (%) or number (95% confidence interval).

184 (89.3%) were tested by the manual method. The IS one-dilution titer threshold for most of these products was 50, while a threshold of 64 was also used on some group O SDP units (Table 1). The performance characteristics of the combined manual and automated IS one-dilution buffered gel card test methods are shown in Table 2. Overall, the buffered gel card had an accuracy of 0.90 (95% CI, 0.87–0.93) (Table 2). Of the 412 antibodies tested, there were 31 (7.5%) false-positive results and 9 (2.2%) false-negative results detected (Table 2). In the reference method, there were a variety of different agglutination patterns observed in the false-positive results, with the majority (16 of 31; 51.6%) demonstrating positivity only at the lowest two dilutions below the threshold dilution (Table 3). Likewise, there were different patterns of agglutination observed in the false-negative results, with 7 of 9 (77.8%) occurring when the reference titer method reacted positively at the titer threshold itself but not at any of the higher dilutions.

IS one-dilution titer performed by automated microplate

In total, 98 antibodies from 49 group O WB units were tested by both the laboratory's routine automated microplate IS one-dilution method and by the reference tube titer method that included a 1-hour RT incubation. The titer threshold for all of these WB units was 50. The performance characteristics of this automated microplate IS one-dilution titer test are shown in Table 2. Overall, this method had an accuracy of 0.90 (95% CI, 0.82–0.95) (Table 2). Of the 98 antibodies tested, there were 3 (3.1%) false-positive results and 7 (7.1%) false-negative results detected with this method (Table 2). There was a variety of agglutination patterns observed among the false-positive results, with one antibody (1 of 3; 33.3%) not demonstrating agglutination at any of the dilutions in the reference titer method, while two other antibodies (2 of 3; 66.7%) showed positivity at both dilutions below the titer threshold (Table 3). All of the false-negative results obtained using this automated method

TABLE 3. The agglutination patterns observed in the reference titer method that included a 1-hour RT incubation for the false-positive and false-negative results that were obtained with the three IS one-dilution titer methods. The percent of the antibodies are listed as a percentage of the total number of false-positive and false-negative results for each titer method.

		Reference titer method					
Immediate spin one-dilution titer method	Nature of immediate spin one-dilution titer method error	−2 dilution	−1 dilution	Threshold dilution	+1 dilution	+2 dilution	Number of antibodies (%)
Manual tube	False positive	pos	pos	neg	neg	neg	2 (100)
	False negative	pos	pos	pos	neg	neg	24 (75)
	False negative	pos	pos	pos	pos	neg	8 (25)
Manual and automated buffered gel card	False positive	neg	neg	neg	neg	neg	2 (6.5)
	False positive	pos	neg	neg	neg	neg	13 (41.9)
	False positive	pos	pos	neg	neg	neg	16 (51.6)
	False negative	pos	pos	pos	neg	neg	7 (77.8)
	False negative	pos	pos	pos	pos	neg	2 (22.2)
Automated microplate	False positive	neg	neg	neg	neg	neg	1 (33.3)
	False positive	pos	pos	neg	neg	neg	2 (66.7)
	False negative	pos	pos	pos	neg	neg	2 (28.6)
	False negative	pos	pos	pos	pos	neg	5 (71.4)

Pos = positive, neg = negative.The dilutions are described relative to the institution's threshold dilution: for example, at an institution that uses a titer threshold of 50 (1:50 dilution), the −2 dilution would be 1:16, the −1 dilution would be 1:32, the +1 dilution would be 1:64, and the +2 dilution would be 1:128. At an institution that uses a critical titer of 100 (1:100 dilution), the −2 dilution would be 1:32, the −1 dilution would be 1:64, the +1 dilution would be 1:128, and the +2 dilution would be 1:256, etc.

demonstrated agglutination at the titer threshold dilution in the reference titer method, with some (5 of 7; 71.4%) also demonstrating agglutination at the next higher dilution (Table 3).

DISCUSSION

This study demonstrated that IS one-dilution titer tests performed by the manual tube, the manual and automated buffered gel card, and the automated microplate had an accuracy rate of 0.90 or greater when compared to a reference tube titer method that included a 1-hour incubation. In fact, among all of the false-negative results with all methods in this study, the errant positivity that was detected in the reference method occurred at dilutions that were either at or one dilution higher than the titer threshold. This finding likely reflects cases where the donor's antibody titer was very close to the threshold itself; extra sensitivity might have been obtained by having performed the 1-hour incubation in the reference method, which led to detecting the agglutination that was missed in the three IS one-dilution methods. The finding that the units that produced false-negative results in these IS one-dilution titer methods generally had titers that were very close to the threshold is reassuring because when a false-negative result occurs and a unit with a higher-than-intended antibody titer is issued in an incompatible manner, the recipient is not usually going to receive a unit with an especially high titer.

The manual and automated IS one-dilution buffered gel card titer method was somewhat less accurate compared to the IS one-dilution tube method, with an accuracy rate of 0.90. Furthermore, this technique produced a significant fraction of false-positive results, 7.5% of all antibodies tested by this method, which contributed to its lower specificity (0.87) and positive predictive value (0.85). In practice, false-positive results would not expose the patient to hemolytic risk from a unit with a high titer but would have led to unnecessarily excluding eligible plasma-containing blood product donations from use in potentially incompatible situations. However, the buffered gel card test is typically more sensitive than the tube method, generally demonstrating agglutination at higher dilutions compared to the tube method when the same plasma samples are tested.[7] Thus, some of these false positives, especially when the two dilutions below the titer threshold were positive, might actually have been true positives as a result of the buffered card's higher sensitivity despite the 1-hour incubation performed in the reference tube titer method.

The main limitation of this study was the relatively small sample size, especially with respect to the automated microplate IS one-dilution titer method. However, the 95% CI for most of the performance measurements in Table 2 are narrow, which indicates the veracity of these results. Furthermore, the overall relatively small number of units tested might have obscured the true incidence of uncommon events that were not detected in this study, such as false-negative results in the IS one-dilution titer methods that produced agglutination at both of the titers above the threshold titer. Thus, caution is advised when interpreting these results. Also, a recent study of the American civilian hospitals that are using LTOWB units for resuscitating trauma patients, along with a hospital in Norway, demonstrated that the majority of the respondents used a titer threshold of either less than 200 followed by less than 256 and less than 50.[2] Only titer thresholds of less than 50 and 64 or less were tested in this study, and while it is unlikely that these manual and automated tests would have demonstrated different performance characteristics at higher titer thresholds, this possibility cannot be excluded. Similarly, the majority of the units tested in this study were group O WB; thus, it is not clear if the performance characteristics found in this study can be extrapolated to other plasma-containing products. Finally, as the technologists performing the titers were not blinded to the results of the IS and reference methods, it is possible that they might have been aware of the corresponding result in the other titer method when scoring their results. This could have introduced bias when scoring the agglutination strength. However, given the large number of tests performed and the multi-institutional nature of this study, it is unlikely that this bias would have significantly affected the overall results.

These data suggest that the IS one-dilution titer test using either manual tube or manual and automated buffered gel card methods without an incubation period is suitable for the identification of high-titer units, thereby eliminating the need for the transfusion service or blood center to perform an extended incubation during titer testing.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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