Report

Warm autoantibodies: time for a change

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Routine adsorption procedures to remove autoantibodies from patients' serum often require many hours to perform. This time-consuming process can create significant delays that affect patient care. This study modified the current adsorption method to reduce total adsorption time to 1 hour. A ratio of one part serum to three parts red blood cells (RBCs; 1:3 method) was maintained for all samples. The one part serum was split into three tubes. Each of these three aliquots of serum was mixed with one full part RBCs, creating three adsorbing tubes. All tubes were incubated for 1 hour with periodic mixing. Adsorbed serum from the three tubes was harvested, combined, and tested for reactivity. Fifty-eight samples were evaluated using both the current method and the 1:3 method. Forty-eight (83%) samples successfully adsorbed using both methods. Twenty (34.5%) samples contained underlying alloantibodies. The 1:3 method demonstrated the same antibody specificities and strengths in all 20 samples. Eight samples failed to adsorb by either method. The 1:3 method found previously undetected alloantibodies in three samples. Two samples successfully autoadsorbed but failed to alloadsorb by either method. The 1:3 method proved to be efficient and effective for quick removal of autoantibodies while allowing for the detection of underlying alloantibodies. ***Immunohematology* 2013;29:5–10.**

Key Words: autoantibody, alloantibody, autoadsorption, alloadsorption

Red blood cell (RBC) autoantibodies, when present in the serum of a patient, will react with the patient's RBCs as well as with all normal RBCs. These autoantibodies have the potential of masking the presence of underlying clinically significant alloantibodies. When a patient with warm autoantibodies in the serum is in urgent need of an RBC transfusion, the time-intensive adsorption process to remove autoantibodies can adversely impact patient care. The current published adsorption procedure[1] (current method) used by reference laboratories and transfusion services can require 4 to 6 hours to complete and is not guaranteed to successfully remove the autoantibodies.

A less time-consuming alternative is needed to expedite the adsorption process and, at the same time, effectively remove the warm autoantibodies. One method would be to increase the RBC-to-serum ratio in an attempt to more effectively remove autoantibodies. Increasing the ratio of RBCs provides more antigen sites to adsorb the autoantibodies; however, this method has been reported to cause dilution of the serum.[1]

This study evaluated a modified, less time-consuming adsorption procedure that could potentially yield results comparable to those produced by the current method. The modified adsorption procedure involved adjusting the initial serum-to-RBC volumes to a 1:3 ratio (1:3 method) and thus making more antigen sites available to adsorb warm autoantibodies.

Materials and Methods

Samples

A total of 58 patient samples known to contain warm autoantibodies were obtained at random. Samples were required to have exhibited autoantibody reactivity and to have had either autologous or allogeneic adsorptions performed using current methods. Three types of samples were used for comparison testing: (1) those that successfully autoadsorbed or alloadsorbed and demonstrated no underlying alloantibodies; (2) those that successfully autoadsorbed or alloadsorbed and demonstrated underlying alloantibodies; and (3) those that did not successfully autoadsorb or alloadsorb and required allogeneic adsorption using 20 percent polyethylene glycol (PEG) prepared in-house (Sigma-Aldrich, St. Louis, MO).

Ficin Treatment of Adsorbing Cells

Ficin-treated allogeneic RBCs for warm adsorption were selected to match the patient's Rh, K, Kidd, and Ss phenotype. The volume of RBCs was determined accordingly to yield the required volume; a 3-mL aliquot was generally used. RBCs were obtained from designated "adsorbing units." The adsorbing RBCs were washed once with 0.9 percent normal saline in a large 16 × 100-mm test tube. The tube was centrifuged to pack the cells, and as much supernatant saline was removed as possible. The washed cells were treated with 1 percent ficin prepared in-house (MP Biomedicals, Solon, OH) in the ratio of 0.5 mL of ficin to 1 mL of cells. The tube was mixed several times by inversion and incubated at 37°C for 15 minutes with periodic mixing. The cells were washed three times with large volumes of saline. For the last wash, the tube was centrifuged for 10 minutes without a centrifuge brake to avoid disturbing the RBC-saline interface. As much supernatant saline as possible was removed to prevent subsequent dilution of serum.

Adsorption Using the Current Published Method

All samples selected for this study had adsorptions performed using the current method[1] (Fig. 1). Equal volumes of patient serum and ficin-treated adsorbing RBCs were mixed and incubated at 37°C for 30 minutes to 1 hour with periodic mixing. The tube was centrifuged for 5 minutes, and the one-time adsorbed serum was harvested. Testing for adsorption effectiveness was performed in the same phases for which neat serum demonstrated reactivity and included the following: low-ionic-strength saline (LISS)-37°C (ImmuAdd, Low Ionic Strength Medium; Immucor, Norcross, GA), LISS-antihuman globulin (AHG), and PEG-AHG. Tubes were incubated at 37°C for 15 minutes for PEG and 20 minutes for LISS-AHG. After washing four times with saline, two drops of anti-IgG (Immucor) were added to each tube, and the tubes were centrifuged and read for agglutination. Testing that showed reactivity was followed with additional adsorptions. Adsorption was repeated by transferring the one-time adsorbed serum to another fresh aliquot of ficin-treated RBCs for a second adsorption. If necessary, a maximum of three total adsorptions were performed.

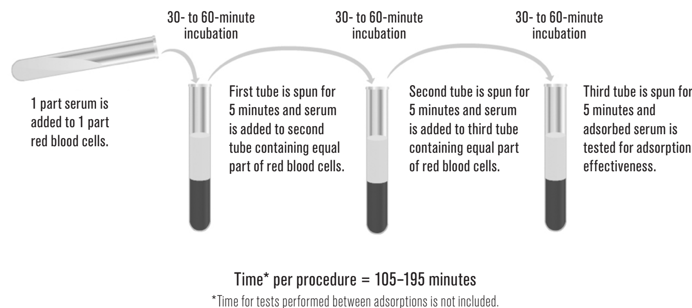

Fig. 1 Current adsorption procedure.

Adsorption Using the Modified 1:3 Method

Adsorption using the modified method was similar to that using the current method except the initial serum-to-cell ratio was modified to a ratio of one part patient's serum to three parts RBCs (1:3 method; Fig. 2). The same adsorbing RBCs used in the original case workup, if adsorbed with allogeneic RBCs, were ficin-treated and dispensed into three separate 12 × 75-mm test tubes. Using a plastic Pasteur pipette, for every three drops of RBCs, one drop of patient's serum was added to yield a ratio of one part serum to three parts RBCs in each tube (1:3 ratio). The cell-serum mixture was mixed and incubated at 37°C for 1 hour with mixing every 10 minutes. An hour of incubation was allowed to best mimic the total adsorption time possible for a routine three-time adsorption.

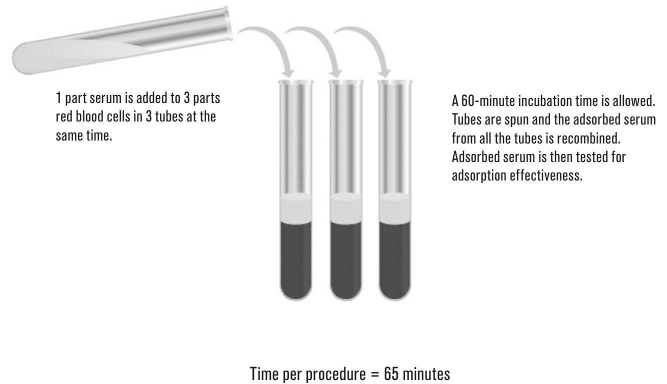

Fig. 2 Modified 1:3 adsorption procedure.

The three tubes were centrifuged for 5 minutes, and the adsorbed serum from all three tubes was combined into a single test tube. Three separate adsorbing tubes, as opposed to only one, were created to best mimic the adsorption conditions used when performing a three-time adsorption following the current published method. Adsorptions using the modified 1:3 method were performed within 1 to 9 days after the original antibody workup (average, 3 days). Samples were stored at 2° to 4°C if testing was to be performed within 5 days. Samples were frozen if testing was to be performed longer than 5 days after the original antibody workup.

Testing of Adsorbed Serum from the 1:3 Method

The adsorbed serum was tested against screening cells if the original adsorbed patient sample demonstrated no underlying alloantibodies and against the selected cell panel used for the original antibody workup if the original patient sample demonstrated underlying alloantibodies. If alloantibody reactivity was detected with screening cells, a full panel and selected cells were tested to make identification. Testing was performed in the same phases that showed reactivity in the original case and included the following: LISS-37°C, LISS-AHG, and PEG-AHG. The effectiveness of the 1:3 method was then compared with previous results obtained from standard adsorption testing.

Statistics

Adsorption results of the 1:3 method were compared with those of the current method. If present, reactivity of each alloantibody in the adsorbed serum was scored for each method using the published scoring system.[1] Data were statistically analyzed using the paired *t* test. The level of significance was established at a probability value of less than 0.05.

Results

Results of the 1:3 method showed 48 of 58 samples (83%) successfully adsorbed, matching the current method (Table 1). Of those 48 samples with successful adsorptions, 20 (34.5%) were known from previous testing to contain alloantibodies. Eight samples failed to be fully adsorbed by either method. Three samples (26, 27, and 28) demonstrated underlying alloantibodies (two anti-E, one anti-f) with only the 1:3 method. Two samples (57 and 58), which previously were successfully adsorbed using autologous RBCs, failed to adsorb using allogeneic RBCs with both the current and 1:3 methods on parallel testing. Two samples (30 and 39) contained underlying IgG antibodies of unknown specificity. In the 20 samples known to contain underlying alloantibodies, the 1:3 method demonstrated the same antibody specificities and comparable reaction strengths as the current method ($p = 0.82$), with one sample (38) showing a stronger reaction by the 1:3 method than by the current method (Table 2). Sample 30 was reactive 1+ LISS-AHG with three of nine panel cells. Sample 39 was reactive 2+ LISS-AHG and PEG-AHG with four of eight panel cells.

Discussion

An important component of pretransfusion testing is to detect clinically significant alloantibodies.[2] Patients with warm autoantibodies in their serum present a unique and challenging problem because the autoantibodies are broadly reactive, reacting with almost all RBCs tested. Warm autoantibodies are the most common cause of autoimmune hemolytic anemia (AIHA), with the incidence of these antibodies increasing with patient age.[3] Although hemolytic transfusion reactions can occur when patients with clinically significant alloantibodies are transfused with RBCs carrying antigens corresponding to the alloantibodies,[4] acute reactions are unlikely when RBC incompatibility is caused by autoantibody alone. Survival of transfused RBCs is generally the same as survival of the patient's own RBCs, and transfusion can be expected to have significant temporary benefit.[3,5,6]

Patients with AIHA can have autoantibodies present in their serum. Warm autoantibodies can cause serologic anomalies including spontaneous agglutination that can result in discrepant ABO and Rh testing. More importantly, warm-reactive autoantibodies can mask the presence of clinically significant alloantibodies. Published data indicate that alloantibodies were detected in 209 of 647 serum samples (32%) of patients with AIHA.[7] Undetected alloantibodies may cause increased hemolysis after transfusion, which can be falsely attributed to an increase in the severity of AIHA.[3,8] Furthermore, although fatalities caused by undetected clinically significant alloantibodies have declined in recent years,[9] the detection of these alloantibodies is still necessary to prevent serious outcomes. When blood transfusion is ordered for a patient with autoantibodies in the serum, specialized serologic testing including adsorption studies, patient phenotyping, and eluate testing are helpful.[10] A knowledge of the patient's complete phenotype is useful to predict which clinically significant alloantibodies can potentially be present in the patient's serum.

One of the most important testing procedures for a patient with AIHA, especially if the patient has a history of pregnancy or transfusion, is adsorption testing to remove autoantibody from the patient's serum and allow for the detection and identification of clinically significant alloantibodies.[1] Adsorption using autologous RBCs is the best procedure to detect clinically significant antibodies. However, autoadsorption should not be performed on samples from patients who have been recently transfused because transfused donor RBCs might adsorb alloantibodies, resulting in a falsely negative test result.

For patients with recent transfusions, the use of allogeneic RBCs is helpful in adsorbing autoantibodies, leaving behind alloantibodies in the adsorbed serum.[1] If the patient's phenotype is known, one allogeneic adsorbing cell can be selected to match the patient's phenotype.[1] The selection of cells is made easier by enzyme treating the allogeneic absorbing cells to destroy the MNS and Duffy antigens.[1] When the patient's phenotype is unknown, differential adsorption can be performed using group O RBCs of three different Rh phenotypes: R_1R_1, R_2R_2, and rr; one cell should lack the Jk^a antigen, and another should lack the Jk^b antigen.[1] Aliquots from each Rh phenotype are prepared and three separate adsorptions are performed, one with each Rh phenotype. Adsorbed serum from each adsorption can only be used to rule out alloantibodies corresponding to the antigens lacking on each adsorbing RBC phenotype. For example, R_2R_2 Jk(a–b+)-adsorbing RBCs can be used to rule out alloantibodies specific for C, e, f, and Jk^a and not D, E, c, or Jk^b.

Adequate testing to detect alloantibodies in the serum of a patient with autoantibodies may take 4 to 6 hours. Adsorption testing using the current method[1] is time consuming and often results in delay of patient transfusion. Also, should routine allogeneic adsorption fail to remove alloantibody reactivity, a PEG-allogeneic adsorption should be performed.[11] PEG-allogeneic adsorption is a faster adsorption method involving

Table 1. Summary of data from all samples tested

Sample	Autologous adsorption	Allogeneic adsorption	Number of adsorptions required	Underlying alloantibodies detected by current method	Underlying alloantibodies detected by 1:3 adsorption method	1:3 method successful at removing autoantibody reactivity?
1		X	1	None	None	Yes
2		X	2	None	None	Yes
3		X	2	None	None	Yes
4		X	3	None	None	Yes
5	X		2	None	None	Yes
6	X		1	None	None	Yes
7	X		3	None	None	Yes
8		X	3	None	None	Yes
9	X		1	None	None	Yes
10		X	1	None	None	Yes
11		X	1	None	None	Yes
12		X	2	None	None	Yes
13	X		2	None	None	Yes
14	X		1	None	None	Yes
15		X	3	None	None	Yes
16	X		1	None	None	Yes
17	X		2	None	None	Yes
18	X		1	None	None	Yes
19	X		2	None	None	Yes
20	X		1	None	None	Yes
21	X		2	None	None	Yes
22		X	1	None	None	Yes
23	X		2	None	None	Yes
24	X		3	None	None	Yes
25		X	3	None	None	Yes
26	X		3	None	Anti-E	Yes
27		X	3	None	Anti-f	Yes
28		X	2	None	Anti-E	Yes
29	X		3	Anti-E	Anti-E	Yes
30		X	3	Anti-K, unknown IgG	Anti-K, unknown IgG	Yes
31		X	3	Anti-E	Anti-E	Yes
32	X		1	Anti-E	Anti-E	Yes
33		X	2	Anti-E	Anti-E	Yes
34		X	3	Anti-E	Anti-E	Yes
35		X	3	Anti-E	Anti-E	Yes
36		X	3	Anti-E	Anti-E	Yes
37		X	2	Anti-Jk^a	Anti-Jk^a	Yes
38		X	1	Anti-E	Anti-E	Yes
39		X	3	Unknown IgG	Unknown IgG	Yes
40		X	1	Anti-S	Anti-S	Yes
41		X	2	Anti-K	Anti-K	Yes
42		X	1	Anti-E	Anti-E	Yes
43		X	1	Anti-E, -Jk^b, -S	Anti-E, -Jk^b, -S	Yes
44		X	2	Anti-E, -C	Anti-E, -C	Yes
45		X	1	Anti-E	Anti-E	Yes
46		X	2	Anti-E	Anti-E	Yes
47		X	1	Anti-C, -E	Anti-C, -E	Yes
48	X		2	Anti-C, -K, -S	Anti-C, -K, -S	Yes
49		X (PEG)	2	None	NA	No - required PEG adsorption
50		X (PEG)	2	None	NA	No - required PEG adsorption
51		X (PEG)	2	None	NA	No - required PEG adsorption
52		X (PEG)	3	Unknown IgG	NA	No - required PEG adsorption
53		X (PEG)	3	Anti-C, -K, -Jk^b, -M, -S	NA	No - required PEG adsorption
54		X (PEG)	3	None	NA	No - required PEG adsorption
55		X (PEG)	3	None	NA	No - required PEG adsorption
56		X (PEG)	2	None	NA	No - required PEG adsorption
57	X		2	None	NA	No - only autologous adsorption successful
58	X		3	Anti-s	NA	No - only autologous adsorption successful

PEG = polyethylene glycol.

Table 2. Comparison of reactivity of alloantibodies in adsorbed serum using current and 1:3 methods

Sample	Alloantibodies: current method	Reaction*	Score	Alloantibodies: 1:3 method	Reaction*	Score
29	Anti-E	1+	5	Anti-E	1+	5
30	Anti-K Unknown IgG	1+ 1+	5 5	Anti-K Unknown IgG	1+ 1+	5 5
31	Anti-E	1+	5	Anti-E	1+	5
32	Anti-E	3+	10	Anti-E	3+	10
33	Anti-E	3+	10	Anti-E	3+	10
34	Anti-E	2+	8	Anti-E	2+	8
35	Anti-E	2+	8	Anti-E	2+	8
36	Anti-E	3+	10	Anti-E	3+	10
37	Anti-Jk^a	2+	8	Anti-Jk^a	2+	8
38	Anti-E	1+	5	Anti-E	2+	8
39	Unknown IgG	2+	8	Unknown IgG	2+	8
40	Anti-S	2+	8	Anti-S	2+	8
41	Anti-K	2+	8	Anti-K	2+	8
42	Anti-E	2+	8	Anti-E	2+	8
43	Anti-E Anti-Jk^b Anti-S	2+ 1+ 1+	8 5 5	Anti-E Anti-Jk^b Anti-S	2+ 1+ 1+	8 5 5
44	Anti-E Anti-C	2+ 1+	8 5	Anti-E Anti-C	2+ 1+	8 5
45	Anti-E	3+	10	Anti-E	3+	10
46	Anti-E	2+	8	Anti-E	2+	8
47	Anti-E Anti-C	2+ 2+	8 8	Anti-E Anti-C	2+ 2+	8 8
48	Anti-C Anti-K Anti-S	2+ 3+ 1+	8 10 5	Anti-C Anti-K Anti-S	2+ 3+ 1+	8 10 5

*Reaction based on reactivity at the strongest phase (either low-ionic-strength saline [LISS]-antihuman globulin [AHG] or polyethylene glycol [PEG]-AHG).

adsorption with one part RBCs, one part PEG, and one part serum for 15 minutes up to a total of three adsorptions. After adsorption, four to six drops of PEG-adsorbed serum are used to test with each panel cell originally reactive with neat serum. Additional enhancement media are not needed because of the PEG present in the PEG-adsorbed serum. Although PEG adsorption is a faster procedure than routine allogeneic adsorption, there is the risk that weak alloantibodies will not be detected.[12] Knowing sooner whether PEG adsorption is necessary aids in reducing the overall turnaround time of making blood available for transfusion.

This study showed that the 1:3 method gave results comparable to those of the current adsorption method, and in much less time, for those samples that originally required more than one adsorption. Of the 58 serum samples selected at random for this study, 48 were successfully adsorbed using both the current and 1:3 methods. Of these 48 samples, 20 (34.5%) contained underlying alloantibodies, which is consistent with the average of 32 percent from published data.[7] In all 20 samples with underlying alloantibodies, the 1:3 method demonstrated the same antibody specificities and reaction strengths as the current method, with one sample yielding stronger alloantibody reactivity in the 1:3 method. Eight samples that failed to be adsorbed by the current method also failed with the 1:3 method. The modified 1:3 method detected underlying alloantibodies in three samples that were not detected using the current method. Two samples that successfully adsorbed in previous testing using autologous RBCs failed to adsorb by the 1:3 method using allogeneic adsorbing RBCs. Further parallel adsorption testing using two separate allogeneic adsorbing RBCs showed both samples failed to adsorb using both the current and 1:3 methods. An explanation could not be found in either of the two samples that successfully autoadsorbed, but failed to alloadsorb by routine methods, as to why only autologous adsorption could remove autoantibody reactivity.

Other studies[13–15] reported that reductions in adsorption incubation times to as little as 10 minutes are equally effective as currently accepted standard methods. Possible future studies could combine this 1:3 method with a shortened incubation time to evaluate whether autoantibodies could still be effectively removed without adverse impact on the final results.

Summary

Standard adsorptions can require 4 to 6 hours; the 1:3 method required approximately 1 to 1.5 hours for the entire adsorption process. In conclusion, this study showed the 1:3 method of using one part patient's serum to three parts RBCs to be time efficient as well as effective for quick removal of autoantibodies while allowing for the detection of underlying alloantibodies.

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