Warm Autoadsorption: Are We Wasting Our Time?

Warm Autoadsorption: Are We Wasting Our Time?

Abstract

BACKGROUND: Routine adsorption procedures to remove autoantibodies from patient's serum often require many hours. This time-consuming process can create significant delays impacting patient care. This study modified the current adsorption method to reduce total adsorption time to one hour.

STUDY DESIGN AND METHODS: A ratio of one part serum to three parts RBCs (1:3 Method) was maintained for all samples. The one part serum was split into three tubes. Each aliquot of serum was mixed with one full part RBCs, creating three adsorbing tubes. All tubes were incubated for one hour with periodic mixing. Adsorbed serum from each tube was harvested, combined and tested for reactivity.

RESULTS: Fifty-eight samples were evaluated using the routine method and the 1:3 Method. Forty-eight (83%) cases successfully adsorbed using both methods. Twenty (34.5%) cases contained underlying alloantibodies. The 1:3 Method demonstrated the same antibody specificities and strengths in all 20 cases. Eight cases failed to adsorb by both methods. The 1:3 Method found previously undetected alloantibodies in three cases. Two cases successfully autoadsorbed but failed to alloadsorb by both methods.

CONCLUSION: The 1:3 Method proved to be efficient and effective for quick removal of autoantibodies while allowing for the detection of underlying alloantibodies.

INTRODUCTION

Red blood cell (RBC) autoantibodies, when present in the serum of a patient, will react with the patient's RBCs as well as 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. Current published adsorption procedure[1] (current method) used by reference laboratories and transfusion services can require 4-6 hours to complete with no guarantee of successfully removing 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 the dilution of serum.[1]

This research study evaluated a modified, less time-consuming adsorption procedure which could potentially yield results comparable to the current method. The modified adsorption procedure involved adjustment of the initial serum to RBCs to a 1:3 ratio (1:3 Method) by 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 must have exhibited autoantibody reactivity and have had either autologous and/or allogeneic adsorptions performed using standard methods. Three types of samples were used for comparison testing: 1) those which successfully auto/alloadsorbed and demonstrated no underlying alloantibodies; 2) those which successfully auto/alloadsorbed and demonstrated underlying alloantibodies; 3) those which did not

successfully auto/alloadsorb and required allogeneic adsorption using Polyethylene Glycol (PEG) prepared in-house (Sigma-Aldrich, St. Louis, MO). Samples were either tested or frozen within five days of patient draw to limit antibody degradation.

Ficin Treatment of Adsorbing Cells

Ficin-treated allogeneic RBCs for warm adsorption were selected to match the patient's Rh, K1, Kidd and Ss phenotype. The volume of RBCs was determined accordingly to yield the required volume; a 3-mL aliquot was generally used. The adsorbing RBCs were washed once with 0.9% normal saline in a large 16x100-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% 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 one hour with periodic mixing. The tube was centrifuged for 5 minutes and the one-time adsorbed serum was harvested and tested for reactivity with the original adsorbing RBCs. Two drops of adsorbed serum were mixed with one drop of the original adsorbing RBCs and 2 drops of LISS (ImmuAdd - Low Ionic Strength Medium; Immucor, Norcross, GA). The tube was incubated at 37°C for 20 minutes. After washing four times with saline, two drops of anti-IgG (Immucor, Norcross, GA) were added, the tube was 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 allowed.

Adsorption Using the Modified 1:3 Method

Adsorption using the modified method was similar to the current method except the initial serum to cell ratio was modified to a ratio of one part patient serum to three parts RBCs (1:3 Method) (Fig. 2). Ficin-treated adsorbing RBCs were first added into three separate 12x75-mm test tubes. A plastic Pasteur pipette was used to count and record the number of drops of cells added to each test tube. For every three drops of red cells added, one drop patient 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 ten minutes. The three tubes were centrifuged for 5 minutes and the adsorbed serum from all three tubes combined into a single test tube.

Testing of Adsorbed Serum from the 1:3 Method

The adsorbed serum was tested against screening cells if the original patient sample demonstrated no underlying alloantibodies and against a selected cell panel if the original patient sample demonstrated underlying alloantibodies. Testing was performed in the same phases that showed reactivity as the original case and included: LISS-37°C, LISS-AHG (Anti-Human Globulin) 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 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 $p < 0.05$.

RESULTS

Results of the 1:3 Method showed that 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. Of 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 showing a stronger reaction in the 1:3 Method compared to the current method (Table 2). Eight samples failed to be fully adsorbed by both methods. Three samples demonstrated underlying alloantibodies (two anti-E, one anti-f) only with 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.

DISCUSSION

An important component of pretransfusion testing is to detect clinically significant alloantibodies.[2] Patients with warm autoantibodies in the 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 the serum if all specific antigen sites of the patient's red cells are bound by autoantibodies *in vivo*. 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 (32%) of patients with AIHA.[7] Undetected alloantibodies may cause increased hemolysis following 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 has declined in recent years[12], 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 elution testing are helpful.[9] A knowledge of the patient's complete phenotype is helpful to predict which clinically significant alloantibodies can be potentially 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 detection and identification of clinically significant alloantibodies. Adsorption using autologous RBCs is the best procedure to detect clinically significant antibodies in the patient who has not been recently transfused. However, autoadsorption should not be performed for patients who have been transfused within the past 3 months because a blood sample for adsorption can contain some transfused donor RBCs that might adsorb alloantibodies.

For patients with recent transfusions, the use of allogeneic RBCs is helpful in adsorbing autoantibodies, leaving behind alloantibodies in the adsorbed serum. If the patient's phenotype is known, one allogeneic adsorbing cell can be selected to match the patient's phenotype. The selection of cells is made easier by enzyme treating the allogeneic absorbing cells to destroy the MNS and Duffy antigens. 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.

Adequate testing to detect alloantibodies in a patient with autoantibodies in the serum may take 4-6 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, the final procedure possible is PEG-allogeneic adsorption.[13] PEG-allogeneic adsorption involves adsorption with 1 part RBCs, 1 part PEG and 1 part serum for 15 minutes up to a total of three adsorptions on

average. Although PEG adsorption is a faster procedure than routine allogeneic adsorption, there is also the risk of non-detection of weak alloantibodies.[14] Knowing if PEG adsorption is necessary sooner aids in the overall turn-around-time of blood delivery.

This study showed the 1:3 Method demonstrated comparable results to the current adsorption method and with much less time. 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 cases, 20 (34.5%) contained underlying alloantibodies, which is consistent with the published report of 32%.[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 cases which successfully autoadsorbed, but failed to alloadsorb by routine methods, as to why only autologous adsorption could remove autoantibody reactivity.

Other studies[10,11] reported that reductions in adsorption incubation times, even as little as a ten minute total incubation time, are as equally effective as currently accepted standard methods. Possible future studies could combine this 1:3 Method with an even shorter incubation time to evaluate if autoantibodies could still be effectively removed without adverse impact on the final results.

SUMMARY

Standard adsorptions can require 4-6 hours; the 1:3 Method required approximately one and a half hours for the entire adsorption process. In conclusion, this study showed that the 1:3 Method of

using a 1 part patient serum to 3 parts RBCs to be time-efficient as well as effective for quick removal of autoantibodies while allowing for the detection of underlying alloantibodies.

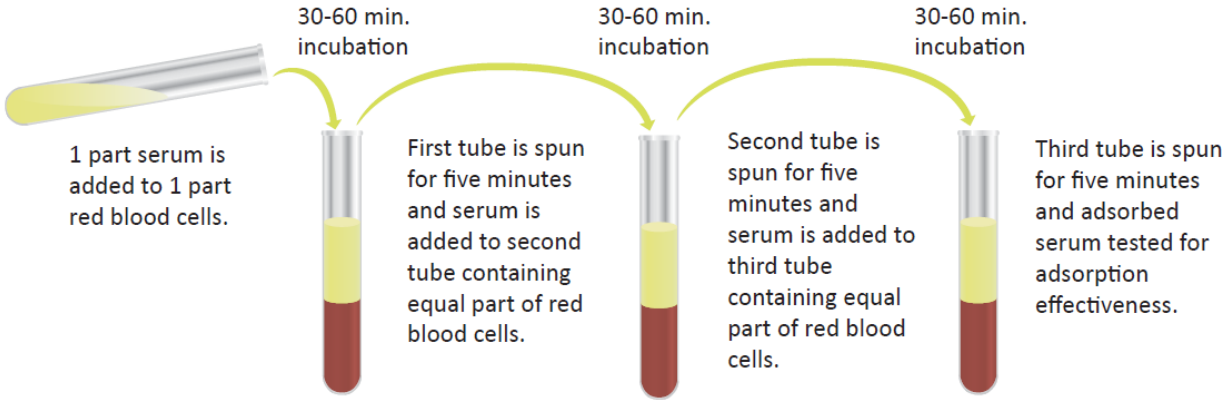
REFERENCES

1. Roback JD, Combs MR, Grossman BJ, Harris T, Hillyer CD. Technical manual. 17th ed. Bethesda, MD: AABB, 2011.
2. Carson TH, ed. Standards for blood banks and transfusion services. 27th ed. Bethesda, MD: AABB, 2011.
3. Petz LD, Garratty G. Blood transfusion in autoimmune hemolytic anemias. In: Immune hemolytic anemias, 2nd ed. New York: Churchill Livingstone, 2004.
4. Jenner PW, Holland PV. Diagnosis and management of transfusion reactions. In: Petz LD, Swisher SN, Kleinman S, eds. Clinical practice of transfusion medicine. 3rd ed. New York: Churchill-Livingstone, 1996: 905-929.
5. Garratty G, Petz LD. Transfusing patients with autoimmune haemolytic anaemia. Lancet. 1993;341:1220.
6. Salama A, Berghofer H, Mueller-Eckhardt C. Red blood cell transfusion in warm-type autoimmune haemolytic anaemia. Lancet. 1992; 340:1515-1517.
7. Branch DR, Petz LD. Detecting alloantibodies in patients with autoantibodies. Transfusion 1999;39:6-10.
8. Petz LD. Blood transfusion in acquired hemolytic anemias. In: Petz LD, Swisher SN, Kleinman S, eds. Clinical Practice of Transfusion Medicine, 3rd ed. New York: Churchill Livingstone, 1996: 469-499.
9. Douglas P. Blackall DP. How do I approach patients with warm-reactive autoantibodies? Transfusion 2011;51:14-17.
10. McConnell G, Kezeor K, Kosanke J, Hinrichs M, Reddy R. Warm Autoantibody adsorption time may be successfully shortened to 15 minutes. (Abstract) Transfusion 2008;48:232A.
11. Fueger J, Hamdan Z, Johnson S. Ten-minute autoantibody adsorptions: They really work!. (Abstract) Transfusion 2008;48:230A.
12. Fatalities Reported to FDA Following Blood Collection and Transfusion: Annual Summary for Fiscal Year 2010. Retrieved February 6, 2012, from

http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/ucm254802.htm

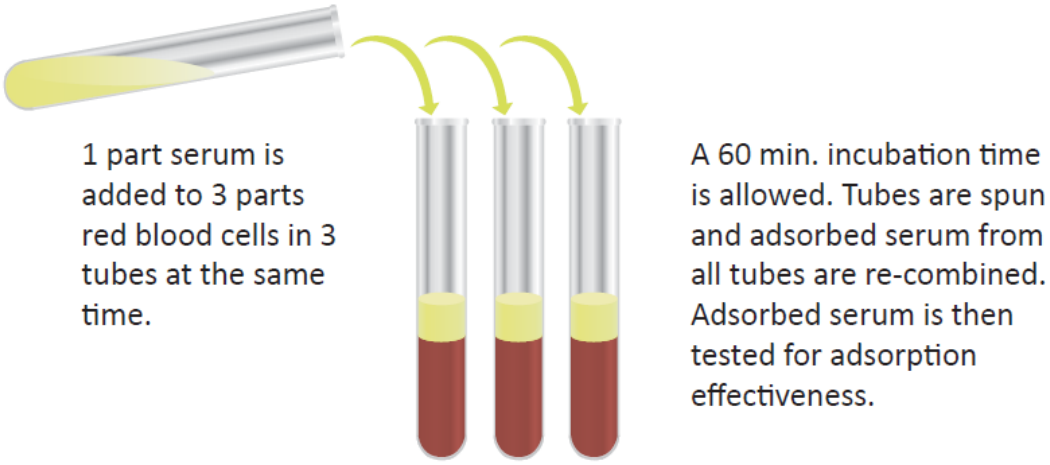
13. C.L. Barron and M.B. Brown. The use of polyethylene glycol (PEG) to enhance the adsorption of autoantibodies. Immunohematology, vol. 13, number 4, 1997. 119-122.
14. W.J. Judd and L. Dake. PEG adsorption of autoantibodies causes loss of concomitant alloantibody. Immunohematology, vol. 17, number 3, 2001. 82-85.

Figure 1. Current Adsorption Procedure not Including Tests between Adsorptions



Time per procedure = 105-195 min.

Figure 2. Modified 1:3 Adsorption Procedure



Time per procedure = 65 min.

Table 1. Summary of testing

Summary of data from all samples tested						
Sample	Autologous Adsorption	Allogeneic Adsorption	Number of Adsorptions required	Underlying Alloantibodies detected with current method	Alloantibodies detected after 1:3 adsorption method	1:3 Method successful at removing autoantibody reactivity?
1		X	1	No	No	Yes
2		X	2	No	No	Yes
3		X	2	No	No	Yes
4		X	3	No	No	Yes
5	X		2	No	No	Yes
6	X		1	No	No	Yes
7	X		3	No	No	Yes
8		X	3	No	No	Yes
9	X		1	No	No	Yes
10		X	1	No	No	Yes
11		X	1	No	No	Yes
12		X	2	No	No	Yes
13	X		2	No	No	Yes
14	X		1	No	No	Yes
15		X	3	No	No	Yes
16	X		1	No	No	Yes
17	X		2	No	No	Yes
18	X		1	No	No	Yes
19	X		2	No	No	Yes
20	X		1	No	No	Yes
21	X		2	No	No	Yes
22		X	1	No	No	Yes
23	X		2	No	No	Yes
24	X		3	No	No	Yes
25		X	3	No	No	Yes
26	X		3	No	Yes (anti-E)	Yes
27		X	3	No	Yes (anti-f)	Yes
28		X	2	No	Yes (anti-E)	Yes
29	X		3	Anti-E	Yes	Yes
30		X	3	Anti-K, Unknown IgG	Yes	Yes
31		X	3	Anti-E	Yes	Yes
32	X		1	Anti-E	Yes	Yes
33		X	2	Anti-E	Yes	Yes
34		X	3	Anti-E	Yes	Yes
35		X	3	Anti-E	Yes	Yes
36		X	3	Anti-E	Yes	Yes
37		X	2	Anti-Jka	Yes	Yes
38		X	1	Anti-E	Yes	Yes
39		X	3	Unknown IgG	Yes	Yes
40		X	1	Anti-S	Yes	Yes
41		X	2	Anti-K	Yes	Yes
42		X	1	Anti-E	Yes	Yes
43		X	1	Anti-E, -Jkb, -S	Yes	Yes
44		X	2	Anti-E, -C	Yes	Yes
45		X	1	Anti-E	Yes	Yes
46		X	2	Anti-E	Yes	Yes
47		X	1	Anti-C, -E	Yes	Yes
48	X		2	Anti-C, -K, -S	Yes	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, -Jkb, -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

Table 2. Comparison of Reactivity of Alloantibodies in the Adsorbed Serum

Sample	Alloantibodies: current method	Reaction*	Score	Alloantibodies: 1:3 Method	Reaction*	Score
29	Anti-E	1+	5	Anti-E	1+	5
30	Anti-K,	1+	5	Anti-K,	1+	5
	Unknown IgG	1+	5	Unknown IgG	1+	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,	2+	8	Anti-E,	2+	8
	-Jk^{b}	1+	5	-Jk^{b}	1+	5
	-S	1+	5	-S	1+	5
44	Anti-E,	2+	8	Anti-E,	2+	8
	-C	1+	5	-C	1+	5
45	Anti-E	3+	10	Anti-E	3+	10
46	Anti-E	2+	8	Anti-E	2+	8
47	Anti-E, -C	2+	8	Anti-E, -C	2+	8
	-C	2+	8	-C	2+	8
48	Anti-C,	2+	8	Anti-C,	2+	8
	-K	3+	10	-K	3+	10
	-S	1+	5	-S	1+	5

* Reaction based on reactivity at the strongest phase