SOUTH CENTRAL
Association of Blood Banks

2019 Sol Haberman Award Winner: Erin Westby, MLS(ASCP)

Review of the PEG Adsorption Technique for Removal of Warm Autoantibodies

Background: The detection and investigation of warm autoantibodies is important for diagnosing unexplained anemia in patients. One of the main goals with evaluating patients with warm reactive autoantibodies is to determine if there is an underlying alloantibody. To eliminate interference from the warm autoantibody, adsorption procedures are performed to remove the autoantibody, allowing detection of alloantibody that may be present in the adsorbed serum.

Literature Review: Numerous adsorption techniques have been used in autologous and allogeneic adsorptions. The PEG adsorption method has been proposed as a fast and effective way to remove autoantibody. This paper reviews studies that compare the different methods to establish if PEG or LISS adsorptions are an adequate and effective method for autoantibody removal.

Conclusions: PEG adsorptions have been proven to decrease the time required to perform these workups.

Introduction

The use of adsorptions to help detect and identify antibodies is a common method used in blood banking. Adsorptions are especially useful in patients with autoantibodies that interfere with routine antibody detection and compatibility testing. Autoantibodies are produced when T regulatory cells (T regs) malfunction within the body. T regs prevent B cells from over producing antibodies, and when this function is lost there is a response to self-antigens, mainly because T regs do not inhibit B cell activity.[1] Several different techniques can remove autoantibodies to help detect underlying alloantibodies, including: (1) untreated adsorptions, (2) PEG or LISS adsorptions, and (3) adsorptions using red cells treated by enzymes (such as ficin or papain) or by a combination of DTT plus enzyme (the ZZAP reagent).

Detection and investigation of warm autoantibodies is important when investigating unexplained anemias. Patients affected by these autoantibodies have a condition known as warm autoimmune hemolytic anemia (WAIHA). The onset of WAIHA can be associated with pregnancy, trauma, infection, and certain underlying diseases. It also can be completely unexplained, what is known as primary (or idiopathic) WAIHA.[1] Most WAIHA patients present with immunoglobulin (usually IgG) and/or complement coating their red blood cells.[1] Complement is part of the innate immune response, and its main mechanism of action is opsonization. Depending on the immunoglobulin class or complement coating the red blood cells, this can cause extravascular hemolysis.[1] During serologic testing, autoantibodies can complicate the identification of alloantibodies by masking their presence. Establishing an efficient adsorption method will result in both time and cost savings, as well as better turn around for patient care. This procedure is critical for any blood bank laboratory that finds autoantibodies during the course of patient testing.

Autoadsorptions, using the patient's autologous red cells, can only be done if the patient has not been recently transfused. Alloadsorption is needed if the patient has been transfused, usually in the past 3 months, or if the patient has severe anemia and the quantity of his/her own RBCs is insufficient to be used for the adsorption procedure.[1] Typically, alloadsorption is performed by adding patient serum or plasma to red cells that are phenotypically matched to the patient. The serum/plasma and red cell mixture is then incubated at 37°C for 15-60 minutes. The mixture is agitated periodically to ensure the antibody is adequately adsorbed. After incubation, the mixture is centrifuged, and the adsorbed serum is transferred to a clean test tube. Multiple adsorptions may be needed to remove the autoantibody if it is strongly reactive. Usually this would be determined by the strength of the DAT, e.g., 1+ reactivity would equate to 1 adsorption needed.

ZZAP adsorptions also require additional time to treat the red cells. Two volumes of ZZAP is added to one volume of red blood cells and incubated at 37C for 30 minutes. The ZZAP-treated packed red blood cells are then washed three to four times before the addition of patient serum/plasma. Next, an equal volume of patient serum/plasma is added to the ZZAP-treated red blood cells and incubated 30-60 minutes at 37C. This can be extremely time-consuming if multiple adsorptions are needed due to having to repeat all the steps. When performing adsorptions in the presence of PEG or LISS, the incubation time can be significantly decreased. Usually, a 1:1:1 (RBC:serum/plasma:PEG) ratio is mixed and incubated for 15 minutes at 37C. If more adsorptions are required, the adsorbed serum/plasma+PEG mixture is added to more adsorbing cells.

This review will compare adsorption techniques for the removal of warm autoantibodies. The hypothesis of this review is that PEG or LISS adsorptions are more efficient and cost

effective than ZZAP adsorptions. The first aim of this review is to determine and compare the efficiency of autoantibody removal between PEG, LISS and ZZAP adsorptions. The second aim is to evaluate the turn-around time for PEG, LISS and ZZAP adsorptions. The third aim is to determine if PEG adsorption can result in cost savings.

Literature Review

Establishing procedures that will reduce costs and improve efficiency of adsorptions to detect underlying alloantibodies is critical to help with the selection of blood components and providing appropriate blood for transfusion in a timely manner. Most autoantibodies react with high incidence RBC antigens, and most of them (67%) have both IgG and complement coating the RBCs.[1] Barron and Brown[2] tested 19 patient samples with warm autoantibodies, 14 of which contained alloantibodies. Using PEG adsorptions, 13 total specificities were detected with the exception of two weak antibodies which were not observed in PEG at all. PEG also showed weaker reactivity in four of the samples compared to papain treated cells. Their results also showed weaker reactivity with papain in five samples and one auto-anti-E that was not detected at all. Judd and Dake[3] tested 11 samples showing comparable results with PEG and ZZAP methods. PEG showed decreased reactivity with three samples and ZZAP showed decreased reactivity in four. Both methods were unable to remove the autoantibody from one sample. Leger and Garratty[4] tested 28 samples, 8 that contained previously identified alloantibodies, in parallel by PEG and ZZAP methods. The results showed that alloantibodies in the PEG-adsorbed sera reacted equal to or greater than those in the ZZAP-adsorbed sera. A previously identified anti-E did not react with PEG or ZZAP in one sample. They then tested 10 more samples in a blind study, not knowing if alloantibodies were present or not, that showed comparable results using

PEG and ZZAP methods. An auto-anti-E was demonstrated with PEG but not with ZZAP. Cid et. al.[5] tested 31 samples with warm autoantibodies using PEG adsorptions and ZZAP adsorptions which detected the same underlying alloantibodies. A modified PEG adsorption method has also been evaluated proving the ability to remove the autoantibodies was comparable to unenhanced adsorptions.[6] Etem et. al.[6] combined 2:1:1 volume (adsorbing cells; untreated or ficin treated: patient serum:PEG) and incubated for 10 minutes. Table 1 summarizes how many samples were tested with each different technique performed. Their results showed the untreated adsorptions did not completely remove the autoantibody from two samples and the modified PEG-enhanced adsorption (with or without enzyme) removed all autoantibodies. The PEG-enzyme adsorption decreased the time to perform the testing as well.[6]

Comparing PEG and LISS studies have also been performed to determine if LISS is an acceptable method for adsorptions. An example of this was a study performed by Das and Chaudhary[7] utilizing PEG and LISS-papain adsorption methods which resulted in concordant results. They also tested both autoadsorptions and alloadsorptions with both methods which table 1 summarizes. Chiaroni et. al.[8] performed a different study with LISS adsorptions compared to papain-treated RBCs and LISS-papain treated cells. All three methods showed similar reactivity, and effectiveness with adsorbing the autoantibody and detecting the underlying alloantibody. An interesting study by Cheng et. al.[9] uses polyclonal; anti-D, anti-c, anti-E, anti-Fy^a, and anti-Jk^a to compare the effectiveness of unenhanced vs. PEG adsorption methods. They used a scoring system where the lower the score, the better the adsorption with antiserum dilutions of 1:1 to 1:32. Their results showed that PEG was more efficient in removing antibodies. The second part of this study was to show the dilution effect of PEG adsorptions. Alloanti-E was used for the

adsorptions and showed there was no dilution effect from addition of PEG on the alloantibodies and the strength of reactivity was the same with 1, 2, and 3 adsorptions.[9]

Maley et. al.[10] found the incidence of red cell alloantibodies underlying pan reactive warm autoantibodies to be 30 to 40 percent. They observed 39 out of 129 samples containing alloantibodies with a broad range of specificities noting that 19 (48.7%) of these were anti-E specificity.

Detection and identification of these underlying alloantibodies is the most important outcome of adsorption methods. However, it is extremely time consuming to identify these alloantibody specificities. Table 1 provides a summary of the different adsorption methods, the mean number of adsorptions required and the mean time required for completion. These studies present data concluding that PEG and LISS adsorptions significantly decreased the time to actually perform the adsorptions. The studies also showed that PEG reduced the number of adsorptions needed.

The cost of Gamma PEG (Immucor, Inc; Norcross, GA) also should be noted which is significantly lower compared to W.A.R.M (Immucor, Inc), a reagent similar to ZZAP. W.A.R.M. costs 2.5 times more than PEG with only 1-2 tests per bottle compared to approximately 10 tests from Gamma PEG. W.A.R.M. also has a 5-day expiration date after the powder is reconstituted. PEG can also be made in a laboratory setting which can greatly decrease the cost of reagent. PEG can be purchased in powder form and reconstituted with phosphate buffer saline. Due to the stability of the mixture, the PEG usually has a long expiration date.

Although these past studies showed PEG adsorptions are an efficient, time and cost saving method, the results are hard to equate because of the different techniques compared and

number of samples tested. Table 2 shows the different techniques with sample sizes performed throughout all the studies previously discussed.

Some studies showed a decrease in reactivity, or the loss of the reactivity completely in some samples,[2,3,4] with PEG adsorptions, while other studies showed PEG enhanced the reactivity when compared to other methods.[2,3,4] One study showed PEG could remove the autoantibody and detect 85% of underlying alloantibodies, and 21% of PEG adsoprtions decreased the reactivity.[2] Papain treated RBCs decreased the reactivity in 26% and 92% of the masked alloantibodies were detected.[2] Judd and Dake[3] demonstrated that PEG adsorptions decreased the reactivity in 27% of samples and ZZAP decreased the reactivity in 36% of the samples tested. Comparable results were also demonstrated with ZZAP-treated cells, enzyme-treated cells, LISS-enzyme, PEG-enzyme and PEG adsorptions.[2-9] The study comparing LISS-papain, papain-treated RBCs and untreated LISS adsorptions showed similar results with all three methods.[8] All studies performed testing PEG vs. unenhanced adsorptions showed PEG was able to remove the autoantibody better to detect the underlying alloantibody.[6,9]

The limitations of alloadsorptions must also be considered. Alloantibody to a high incidence antigen will be adsorbed out with the warm autoantibody and thus, these alloantibodies cannot be identified or ruled out.[11] Also, the more adsorptions needed, the greater chance of diluting the serum resulting in weakened reactivity or loss of reactivity of any underlying alloantibodies. Tsimba-Chitsva and Kezeor[12] stated, “Enzyme treatment of autologous RBCs can cause hemolysis. If the RBCs are hemolyzed by enzyme treatment there may be an insufficient quantity of RBCs remaining to perform adsorptions.” Enzyme treatment can destroy some antigens against which the autoantibody might be directed, thus causing an apparent failure of

the adsorption technique. The technologist's technique, time incubated, and centrifugation can also alter the results.

Discussion

When testing samples from patients with autoantibodies, the primary concern is to detect and identify any clinically significant alloantibodies. Analyzing the current literature has shown great contrast in whether or not PEG will weaken the reactivity of underlying alloantibodies, consistent with precipitation of the antibody. Some studies showed even enzyme and chemically treated red blood cells may weaken the reactivity of the underlying autoantibody. It must be noted that in routine blood bank testing, no one method will be effective to detect all antibodies. Graph 1 shows the comparison of mean time and mean adsorptions with the different techniques performed. PEG with or without enzyme decreased the number of adsorptions and technologist time dramatically. LISS technique shows it does not decrease the number of adsorptions and actually increases the number though.

A practical approach might be to utilize both PEG and chemically modified adsorptions. The literature does support PEG adsorptions dramatically decreases the time required to perform these extensive workups. This should provide cost savings due to reduced technologist time and improve turnaround time to provide the appropriate red blood cell components. Although there is controversy over the use of PEG adsorptions, there is also evidence that the use of these adsorptions can provide benefit to the patient. To better understand the difference between the various adsorption methods, more studies with larger patient samples should be performed to give better conclusive data.

References

1. Harmening D. *Modern Blood Banking & Transfusion Practices.* 6th ed. Philadelphia, PA: F.A. Davis Company; 2012.
2. Barron CL, Brown MB. The use of polyethylene glycol (PEG) to enhance the adsorption of autoantibodies. *Immunohematology*. 1997;13:119-122.
3. Judd WJ, Dake L. PEG adsorption of autoantibodies causes loss of concomitant alloantibody. *Immunohematology.* 2001;17(3):82-85.
4. Leger RM, Garratty G. Evaluation of methods for detecting alloantibodies underlying warm autoantibodies. *Transfusion.* 1999;39(1):11-16.
5. Cid J, Ortín X, Pinacho A, Parra R, Contreras E, Elies E. Use of polyethylene glycol for performing autologous adsorptions. *Transfusion.* 2005;45(5):694-697.
6. Etem ME, Laird-Fyer B, Holub MP, Held JJ, Symington DB, Figueroa D. Allogeneic adsorptions: a comparison of the traditional method with a modified PEG adsorption method. *Immunohematology.* 2010;26:104-108
7. Das SS, Chaudhary R. Utility of adsorption techniques in serological evaluation of warm autoimmune haemolytic anaemia. *Blood Transfus.* 2009;7:300-304.
8. Chiaroni J, Touinssi M, Mazet M, Micco PD, Ferrera V. Adsorption of autoantibodies in the presence of LISS to detect alloantibodies underlying warm autoantibodies. *Transfusion.* 2003;43(5):651-655.
9. Cheng C-K, Wong ML, Lee AW. PEG adsorption of autoantibodies and detection of alloantibodies in warm autoimmune hemolytic anemia. *Transfusion.* 2001;41(1):13-17.
10. Maley M, Bruce DG, Babb RG, Wells AW, Williams M. The incidence of red cell alloantibodies underlying panreactive warm autoantibodies. *Immunohematology.* 2005;21(3):122-125.
11. Tsimba-Chitsva, Bishop S, Kezeor K. Warm autoadsorption with enzyme-treated red blood cells. *Immunohematology*. 2012;28:88-90.

Appendix

Table 1. Summary of study results: Number of adsorptions and procedure time.

Study	Adsorption Method	Number of Samples	Mean # of Adsorptions	Mean Procedure Time (Min)
Barron & Brown[2]	Enzyme Treated Cells	19	2.2	187.9
	PEG, Untreated Cells		1.6	31.6
Judd & Dake[3]	ZZAP Treated Cells	11	2.0	60.0
	PEG, Untreated Cells		2.0	30.0
Leger & Garratty[4]	ZZAP Treated Cells	28	1.3	39.6
	PEG, Untreated Cells		1.3	19.3
	ZZAP Treated Cells	10	2	60.0
	PEG, Untreated Cells		1.5	22.5
Cid et. al.[5]	ZZAP Treated Cells	31	2	118.0
	PEG, Untreated Cells		1.9	28.0
Etem et. al.[6]	Untreated Cells	20	2.1	84.4
	Enzyme Treated Cells	19	2.1	85.0
	PEG, Untreated Cells	20	1.3	27.0
	PEG, Enzyme Treated Cells	19	1.3	27.3
Das & Chaudhary[7]	LISS, Enzyme Treated Cells	Allo 13	3.9	177.7
	PEG, Untreated Cells	Allo 14	1.4	93.6
	LISS, Enzyme Treated Cells	Auto 6	3.0	103.3
	PEG, Untreated Cells	Auto 8	1.1	34.4
Chiaroni et. al[8]	LISS, Untreated Cells	26	2.9	58.0
	Enzyme Treated Cells	123	6.0	180.0
	LISS, Enzyme Treated Cells	50	2.9	57.6
Cheng et. al.[9]	Untreated Cells	16	2.5	150.0
	PEG Untreated Cells		6.0	22.5

Allo=Alloadsorption Auto=Autoadsorption

Table 2: Sample size and techniques compared.

Study	# samples total	Methods						
		Untrt RBCs	Enz-trt RBCs	ZZAP trt RBCs	PEG with untrt RBCs	PEG with enz-trt RBCs	LISS with enz-trt RBCs	LISS with untrt RBCs
Barron & Brown[2]	19		X		X			
Judd & Dake[3]	11			X	X			
Leger & Garratty[4]	38			X	X			
Cid et. al.[5]	31			X	X			
Etem et. al.[6]	78	X	X		X	X		
Das & Chaudhary[7]	41				X		X	
Chiaroni et. al.[8]	16			X	X			
Cheng et. al.[9]	199		X				X	X

Untrt = Untreated; Trt = Treated; Enz = Enzyme treated

Graph 1: Data on the mean adsorption and time required by each individual technique