2019 Sol Haberman Award Winner
T/S | Oral

Review of the PEG Adsorption Technique for Removal of Warm Autoantibodies

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BACKGROUND/CASE STUDIES: 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.

CONCLUSION: 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.

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T/S | Oral

Does external temperature monitoring risk premature discard of red cell units?

Author: Dr Frances Compton

BACKGROUND: Objective The “30-minute rule” is often used as a general guideline for how long a prepared blood component unit can be held at room temperature and still maintain the required shipping temperature of 1-100C. This unwritten rule is often used during shipping preparation and hospital transportation. In our preparation to validate the 30-minute rule, we set out to determine blood component temperature internally as well as externally. As there are limited studies which document the best way to obtain blood unit temperature, we decided to investigate two different methods of temperature measurement.

METHOD: We selected four expired red blood cell (RBC) units to be included in the study. All four units had been stored in our blood refrigerator prior to the study, which is continuously monitored to maintain adequate storage temperature of 1-6oC. We used two of these units for external temperature measurement and the other two units were used for internal temperature measurement. We used validated thermocouple temperature probes to measure the external and internal temperatures of the selected units at five-minute intervals, until all units went out of shipping temperature range (>100C). We cut a small hole in the two internally measured RBC units to insert the probe, which precluded us from using the same units for both external and internal measurements. The other two RBC units were used for external temperature measurements. This was obtained by folding the units in half and inserting the probe into the center of the folded units, keeping the probe tip completely surrounded by the unit bag. The study was conducted in a lab continuously monitored to maintain a temperature of 20-24oC.

RESULTS: While the externally measured RBC units exceeded 10oC at 25 and 30 minutes, the internally measured temperatures did not become out of temperature until 50 minutes (Figure 1).

CONCLUSION: Conclusion The study shows that the 30-minute rule is a good general rule for the length of time that units can stay at room temperature during shipping, and also reveals a significant discrepancy between the measured external and internal measured temperatures. Internal temperature measurements stayed in-temperature for much longer than external temperature measurements. This supports the general transfusion service practice of accepting RBCs back in the inventory if retuned within 30 minutes for in hospital use. Furthermore, if transfusion services discard units according to external temperature measurement devices, they may be discarding in-temperature units which are not being measured accurately. Unfortunately, measuring blood units internally is not feasible for clinical practice, but it may be a better approach when validating future practices.

T/S | Oral

Validation of Automated DNA Extraction from Buccal Swab Samples

Author: Katrina Billingsley, MSTM, SBB

BACKGROUND: Extraction of DNA is most commonly performed on white blood cells found in whole blood, EDTA samples. However, there are occasions when whole blood is not a viable option to obtain sufficient DNA for testing, including patients with extremely low white cell counts or those who are post stem cell transplant. DNA extraction from buccal swabs can become an alternative sample source; however buccal swabs often yield very low DNA concentrations and poor purity ratios. If customary sample acceptance criteria of concentrations ≥10ng/µl and purity absorbance A260/A280 ratios of 1.6 – 2.2 are applied to these samples, many would be deemed unfit for DNA testing

STUDY DESIGN/METHODS: DNA extraction from buccal swabs was performed on 27 samples. Swabs were processed using the extraction protocol supplied by QIAGEN for the QIAcube (QIAamp DNA Blood Mini - Buccal swab). Extracted DNA was analyzed for purity and concentration using spectrophotometric technology (NanoDrop, Wilmington, DE). All samples were amplified according to the test method selected. Twenty-two gDNA samples were amplified using primers specific for HGH and visualized on an electrophoresis gel. The remaining five gDNA samples were tested on a microarray assay..

RESULTS/FINDINGS: Of the 27 samples tested, 12 yielded A260/A280 ratios ranging from 0.55 – 1.5 (1.6 – 2.2 preferred) with 21 of 25 samples yielding a concentration <10ng/µl (≥10ng/µl preferred). Regardless of purity and concentration values, all 27 samples yielded acceptable results from their respective PCR tests.

CONCLUSION: Using the QIAcube protocol for buccal swab extraction yields DNA of sufficient quality for PCR processing despite obtaining low purity and quantity readings. This validation demonstrates that conventional sample acceptance criteria may be too strict for buccal swab extraction as all of these samples produced acceptable results upon PCR amplification.

n=27	ng/µl (≥10 preferred)	A260/A280 ratio (1.6-2.2 preferred)
Min	0.6	0.55
Max	35.8	2.22
Mean	7.4	1.51
Median	5.4	1.56
Mode	4.9	1.46

T/S | Oral
Title: Evaluating the Risk of Infectious Disease in Blood Transfusion Safety

Authors: Jessica Peel MLS(ASCP) SBB

BACKGROUND/CASE STUDIES: Emerging infectious diseases (EID) have been a constant worry since the beginning of the modern era of transfusion medicine. EID's can be sub divided into three main categories: established infectious diseases that have been prevalent for an extended period, newly emerging infectious disease, and reemerging infectious diseases that have historically infected humans but continue to reappear due to resistant forms. Since infectious diseases are closely dependent on the nature of human behaviors and lifestyles, it is important to understand how infectious diseases are acquired and how to successfully break the cycle of infection. A risk analysis model was developed to properly evaluate the perceived risk of emerging infectious diseases and provided a clear idea of the best methods to curtail continued transfusion of infectious diseases.

STUDY DESIGN/METHODS: The search strategy focused on information relating to emerging infectious diseases relevant to the safety of successful blood transfusions. The selection criteria for research information and values focused on original research articles obtained from the AABB web site. Data extracted includes characteristics which are attributed to the epidemiology of infectious disease and blood transfusion safety such as: disease impact on populations, prevalence of infection in populations, asymptomatic pathogenesis (days, months, years), disease severity (low, moderate, high), transfusion transmissibility (unknown, plausible, definite), and mortality (low, moderate, high). This proposed approach aims to highlight the changing paradigm of significant transfusion pathogens by applying probability inversions to the value of perceived risk of current emerging infectious diseases relevant to blood transfusion.

RESULTS/FINDINGS: A risk assessment model was constructed to allow for the evaluation of the risk of selected emerging infectious disease per disease characteristic per expression level in the population. The model produced results showing that prevalence in the population and transfusion transmissibility are the most important drivers of risk at 75% importance. The next important risk factors are disease impact on the population and disease transmission during asymptomatic disease phase demonstrating

a 63% risk importance. The model succeeded in serving as a useful screening tool to interpret the relative risk of emerging infectious diseases related to transfusion safety.
Conclusion With established disease characteristics, workable risk analysis models can be developed to appropriately rank understood risks of emerging infectious diseases with the hopes of safer blood transfusions. Implementation of new analysis methods in response to infection risks and systems of assessing emerging infectious diseases are also of merit due to the perpetual challenges faced.

Keywords: Risk analysis, successful blood transfusion, emerging infectious diseases, perceived risk.

A | Poster

Title: Blood Management in a Community Hospital

Authors: Rachel Poffinbarger, BS MT (ASCP)

BACKGROUND/CASE STUDIES: Patient Blood Management results in the reduction of product wastage, unnecessary exposure, adverse reactions, and healthcare costs. Establishing transfusion guidelines promotes a better outcome for our patients. A successful program consists of collaboration between proactive pathologists, physicians, and blood bank staff. Majority of results have been shown from large tertiary care centers. We report our successful Blood Management program in a community hospital setting. Criteria for transfusion were established by a corporate blood utilization committee, with heavy emphasis on red blood cell transfusions with a hemoglobin of </= 7.0 g/dl.

RESULTS/FINDINGS: A slogan of "Why give two, when one will do" was promoted to encourage physicians to check transfusion response between units. Nurses and other clinical personnel were educated on the criteria. Transfusion orders were required to be entered electronically, which included the reason for transfusion. A real-time screening process to flag outlying orders were implemented. Cases that fell out of criteria would be reviewed by the pathologist to determine if the transfusion was justified or not. All cases reviewed were included in a monthly transfusion summary, and quarterly blood utilization meetings.

CONCLUSION: Blood utilization committee members include pathology, quality management, medical staff, administration, and nursing leaders. An initial goal was 50% transfusions meeting criteria was set. Over two years, the Blood Management program has surpassed that goal, reaching to 66%, and has been the model for other regional community hospitals within our system.

T/S | Poster

Anti-C, Only Detected in PeGTM, Implicated in a Transfusion Reaction

Author: Jowi McCray, MLS, (ASCP)SBB

BACKGROUND:
An 88-year-old African American female admitted to the hospital with a diagnosis of atrial fibrillation and anemia. The facility reported no known antibody history and no recent transfusion. Antibody detection testing was positive using GEL technique. The sample was referred to the Immunohematology Reference Laboratory (IRL) for antibody identification and 2-unit crossmatch. IRL records indicated the patient had a history of anti-E. Antibody identification revealed a new anti-M. Auto control and direct antiglobulin test were negative. Crossmatch compatible, E-, M- units were provided for transfusion. The hospital reported a transfusion reaction 24-hours later. The patient's temperature, blood pressure and respiration all increased. Hemoglobinuria was also reported.

METHOD
Antibody identification was performed using tube technique with Gamma LO-IONTM (Immucor, Inc., Norcross, GA). Reactions were read at RT, 30-minutes at 37C and indirect antiglobulin test using anti-human globulin (AHG). Tubes were graded after each phase of testing. Tube testing using Gamma PeGTM (Immucor, Inc., Norcross, GA) was incubated 15-minutes at 37C, and read at AHG. Gamma ELU-KITII (Immucor, Inc., Norcross, GA) and reagent antisera were also used in the investigation. A Monocyte Monolayer Assay (MMA) was performed to predict clinical significance of the identified antibodies. Transfusion Reaction (TRXN) investigation includes clerical check, visual inspection for hemolysis, DAT, repeat ABO/Rh on pre- and post-transfusion samples and additional testing as indicated.

RESULTS:
A post-transfusion sample was referred to the IRL for a TRXN investigation. There were no clerical errors; however, hemolysis was present in the post-transfusion plasma. ABO/Rh and crossmatches using LO-IONTM were repeated on the pre- and post-transfusion samples with no discrepancies. The post-transfusion DAT was positive with a negative eluate. Antibody identification on the post-transfusion sample with LO-IONTM was negative. Suspecting a weak antibody further investigation using PeGTM on both samples revealed an anti-C. No additional clinically significant alloantibodies were detected in the pre- or post-transfusion samples using PeGTM

CONCLUSION:
The patient experienced an acute hemolytic transfusion reaction due to anamnestic interaction of anti-C in the patient's plasma against C antigen on the transfused cells. The anti-C was not detected by our routine antibody identification techniques performed pre-transfusion. The MMA confirmed anti-E, -M, and-C were clinically significant.

T/S | Poster

Serendipity: Two Rhnull Study Subjects Who Became Lifelong Friends

Author: Rich Gammon,MD

BACKGROUND: A 77-year-old female was admitted to emergency room with a subdural hematoma requiring 1 plateletpheresis. Her husband reported Rhnull history and stated it was investigated in 1967.1 He confirmed patient had Rhnull friend who was also part of investigation and had previously donated for patient and lived in area. To be prepared for possible red blood cell orders, the patient's sample was sent to immunohematology reference laboratory (IRL). Rhnull phenotype is rare with lack of expression of all Rh antigens. It is associated with stomatocytosis, spherocytosis, increased osmotic fragility, and elevated Na1/K1 ATPase activity.

METHOD: The hospital reported solid phase screen and panel that reacted 4+ with all cells. IRL performed ABO, Rh, DAT, PeG screen, auto control, and RBC antigen phenotype (CcEe; K; FyaFyb; JkaJkb; MNSs). The DCcEe results as well as the frequency and the strength of the antibody reactivity led us to perform four adsorptions with cells selected based on patient's phenotype, to remove the high incidence alloantibody. An eluate was prepared from cells used for adsorption to help identify high incidence antibody. We used untreated cells for first two and ficin treated cells for last two adsorptions. Sample was sent to molecular laboratory for additional testing. Stomatocytes were seen on peripheral smear. (Figure - arrowhead)

RESULTS: Patient was Asub Negative with anti-A1, negative DAT. RBC antigen profile by serology: D-C-E-c-e-; K-; Fy(a-b+); Jk(a+b-); M+N+S+s+. The plasma reacted 4+ with three cell screen at PeG AHG, with negative auto control. Alloantibodies were not detected in adsorbed plasma. Since R1R1 cells were used to adsorb the plasma, anti-D, -C, - e were not excluded; however, upon completion of adsorption, IRL was notified of history of anti-Rh-29. A portion of the sample was sent to another IRL for further molecular investigation. The patient's Rhnull friend had recently donated an autologous unit that was frozen. The unit was tested by HEA beadchip and the predicted RHCE phenotype was (C+E-c-e+). This result together with the donor screening serological ABO RhD result predicted that the donor was A Negative r'r'. Since this result did not match with donor's history, IRL tested cells from unit and they were Rhnull by serology consistent with 1967 study. HEA beadchip package insert indicated that Rhnull may not be detected.

CONCLUSION: Evaluation of Rhnull requires both serological and molecular methods to ensure detection. Adequate genetic testing to identify underlying mutation, amorph or regulator type of *RHCE/RHD* and *RHAG* genes respectively should be performed to correlate the serology. Good communication between patient, hospital and IRL is critical to ensure that appropriate testing is performed. Two Rhnull donors who met over 50 years prior became friends and one a directed donor for the other. 1.Transfusion 1967; 7: 389-90.