Dear WABB Members,

I was delighted to see many of you at the 2003 Fall Seminar. Again, our Education Committee organized a top-notch educational experience for us with outstanding speakers.

I would like to share some items from the Annual Business Meeting with those of you that were unable to be there with us.

- Registration fees for our WABB meetings, workshops and registration must be increased slightly to help in the attempt to balance the budget.
- WABB equipment was rented for a fee and the proceeds were used in the purchase of refurbished Gel equipment.
- The Gel equipment was used for the WABB Spring Workshop and also a cooperative workshop with Marshfield in October.
- The WABB website is up and running at www.wabb.org. Volunteers are needed to help with creative ideas for maintaining and improving the website. Please contact Peggy Schroeder or me if you can offer any ideas or assistance.
- Our newsletter continues to be printed twice a year under the leadership of Judy Snow. Please contact Judy if you wish to contribute to future editions.
- Volunteers are needed for the Education Committee and Board. Our programs are cost effective due to the time these people volunteer. A range of expertise is needed to be successful. Please contact any Board or Education Committee member if you are interested in finding out more about being involved.
- Individual and Institutional Membership in WABB is strongly urged. The organization needs your support to continue. Encourage your co-workers and institutions to become members.
- Board Officers were elected. Treasurer is Jan Weyhmiller from Marshfield Laboratories; Member at Large is Peggy Schroeder from University Hospital in Madison; and President Elect is Dr. John Weiss from the American Red Cross in Madison.
- The WABB Service Recognition Award was presented to Judy Snow for her dedication to the field of blood banking, immunohematology, and transfusion medicine. Judy’s active participation in WABB has been tremendous. Thank-you, Judy, for all that you have done and continue to do for WABB.

WABB has decided to encourage sponsorship of the breaks at the Fall Seminar and our workshops to defray costs for our participants. We appreciate these sponsors and hope that you will thank them when you see their representatives. The names of these sponsors are listed in this newsletter. If your organization would be interested in sponsoring part of a workshop or Fall Seminar, please contact any Education Committee or Board Member.

I look forward to serving WABB as president for 2004 and hope to see many of you at our Fall Meeting.

Sincerely,
Pat Broderick, MT(ASCP)
WABB President

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What’s the Real ABO?
by Judy Fueger, MT(ASCP)SBB Immunohematology Reference Lab, The Blood Center of Southeastern Wisconsin

A sample was referred to our lab for an ABO discrepancy. The patient was a 32-year-old prenatal patient. Historical information provided by the hospital listed the patient only as Rh positive and transfuse O RBCs if needed.

Initial findings were:

<table>
<thead>
<tr>
<th>Anti</th>
<th>Reverse Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>-A</td>
<td>-B</td>
</tr>
<tr>
<td>Immediate Spin</td>
<td>0</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>4+</td>
</tr>
</tbody>
</table>

An ABO discrepancy is present at immediate spin (IS). The patient forward types as an O and reverse types as a B. When the reverse typing is incubated at room temperature (RT), it appears that the patient could be Group O. According to the SOP in our laboratory, a 1+ positive reaction with reverse cells does not meet the criteria for a valid ABO typing. A 3-4+ reaction with reverse cells is the expected and required result. Our next step is to incubate the reverse typing at a lower temperature. It is important to include appropriate controls since incubating at colder temperatures not only enhances ABO antibodies it also enhances cold autoantibodies. Controls verify that positive reactions are due to ABO and not other cold-reactive antibodies. Controls should include at least two examples of the reverse cells of the appropriate ABO type in question (Group B in this case), group O screening cells and an auto control.

The following results were observed:

<table>
<thead>
<tr>
<th>Reverse Cells</th>
<th>Screening Cells</th>
<th>Auto Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>IS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RT</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>18C</td>
<td>W</td>
<td>2+</td>
</tr>
<tr>
<td>4C</td>
<td>4+</td>
<td>2+</td>
</tr>
</tbody>
</table>

Since the screening cells were positive, a cold panel was performed to identify the antibody present at room temperature and below. Anti-P1 was identified at 18C. The patient’s red cells and group B reverse cells phenotyped as P1+. Therefore, the positive reaction with the Group B cells was most likely due to anti-P1 and not anti-B.

Adsorption and elution studies were then performed on the patient’s red cells to look for the presence of weak B antigen. The patient’s red cells were incubated with human anti-B. Controls of a weak subgroup of B and group O cells were also run in parallel with the patient’s sample.

THE WABB WEBSITE has been updated to include a link to the American Association of Blood Banks (AABB). If you or your institution is a member of AABB, you can link from there to other state organizations (including WABB). Even if you are not a member, the AABB website provides a wealth of interesting and timely information for blood bankers. You can also view the summer edition of Blood Bank Update in its entirety on the website. Adobe Acrobat Reader is required, but there is a link to download it if you don’t have it.

The website is best viewed using Internet Explorer 5.0 and higher, or Netscape Navigator 6.0 and higher. If you have difficulty getting into the website, if any of the pages appear unusual, or if you have a comment or suggestion regarding the website, please contact Peggy Schroeder at pe.schroeder@hosp.wisc.edu.

Wisconsin Association of Blood Bank’s 26th Annual Fall Seminar

Date: Wednesday, September 15 and Thursday, September 16, 2004
* Please note dates (Wed-Thur) *

Location: Radisson Hotel, Brookfield WI (Hwy 18 & 94)

Sponsors are a huge help to allow us to keep registration affordable and still be able to offer interesting topics and fascinating speakers. If your organization would like to sponsor a morning or afternoon break please contact us.

Lisa Leszczynski at 414.545.5570
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AABB Assessments in Italy

By Sue T. Johnson, MSTM, MT(ASCP)SBB
Manager, Immunohematology Services
The Blood Center of Southeastern Wisconsin

The mission of the American Association of Blood Banks (AABB) is “to establish and promote the highest standard of care for patients and donors in all aspects of blood banking; transfusion medicine; hematopoietic, cellular and gene therapies; and tissue transplantation”. The AABB also has an International Mission Statement that reads “To coordinate and promote improvements in blood banking and transfusion safety internationally by supporting: 1) the development of national and/or regional standards in blood banking; transfusion medicine; hematopoietic, cellular and gene therapies; and tissue transplantation; and 2) the development of mechanisms for assessing compliance with those standards. Institutions seeking accreditation are provided educational and peer review opportunities of their quality and operational systems by having an AABB assessment performed.

In July of this year, I had the privilege of performing two AABB assessments in Italy. One in Milano, and the other in Sicily. First, in Milan I performed an assessment of the Immunohematology Reference Laboratory (IRL) at the IRCCS Ospedale Maggiore, Centro di Trasfusionale e di Immunologia dei Trapiantati. This was the first IRL from another country seeking accreditation. I had the opportunity to work with Fernanda Morelati, ScD and Paolo Rebulla, MD.

We started by reviewing the institution’s quality systems to ensure they were meeting the intent of the quality Standards. Contrary to blood banking organizations in the United States, they started their pursuit of AABB accreditation by becoming ISO 9000 certified. This ensured that they would have the quality systems in place to meet AABB Standards.

Next, I assessed the technical operations to ensure that operations met the Standards for Immunohematology Reference Laboratories. One of the unique challenges was language. All of their procedures were in Italian! Given my heritage (some of you may remember my middle initial “T” stands for Tringali) and the fact that I took Italian at the University of Wisconsin, I could understand them most of the time. There were a few key words that I needed to translate, including the following:

- Le emazie (red blood cells)
- Globuli rossi (red blood cells)
- Anticorpi (antibody)
- Fisiologica (saline)
- 2 gocce (2 drops)

“IABB Assessments” continued on Page 4

THANK YOU
Judy Snow

I would like to extend my deepest appreciation to the members of WABB for presenting me with the WABB Service Recognition Award. This is such an honor. I have been a member of WABB for greater than 20 years and it has proven to be an invaluable source of education and networking for me. By attending many of the seminars and workshops, I was able to get to know techs working in different cities in transfusion services and reference labs. When you have a problem in the blood bank and you call for help or if you are transferring a patient to a larger facility and need to forward blood bank information it is nice to put a face to a name. We often have speakers from facilities in Wisconsin and it is nice to be able to call them to discuss anything from a patient problem to a “Standards” interpretation. Being a part of the board of directors was really a great honor. The work was minimal and the benefits fantastic. One example of the benefits is being included in the speaker dinners. Interacting with the experts in a rather informal setting is inspiring and lets you see another aspect of blood banking aside from just the technical bench setting. You don’t have to be from a large institution, you don’t have to have an SBB to be involved, you just need a love of blood banking and a desire to get to know other people.

“Thank You” continued on Page 5
Once I had these down, I could understand their procedures. The next challenge for all of us was speaking and understanding Italian and English. Everyone in the lab could understand some English. I would ask a question of one of the techs in English, and either Dr. Morelati would translate in Italian or they would understand me. They would answer in Italian and most of the time I would understand them. Needless to say, by the end of the day we were all exhausted.

One of the interesting differences between their IRL and those in the U.S.A. was the fact that they didn’t have a cell separation technique as required by AABB Standards. These methods are used to separate a patient’s own red cells from transfused red cells. In place of cell separation, they performed more advanced testing, molecular genotyping, on all patient samples. The donor center had a wonderful “deli” for donors that included Italian bread, salami, mortadella, prosciutto, tomatoes with fresh mozzarella cheese, Costoletta Milanese (breaded veal cutlet) sandwiches, fresh fruit, and espresso. Physicians had office hours at the donor center for individuals if they desired consultation after donating. Staffing included internists, psychiatrists, and cardiologists to name a few. Female donors, who often are deferred for low hemoglobins, are only allowed to donate two times per year according to the Italian Ministry of Health, the governmental agency similar to the F.D.A.

I then traveled to the U.S. Naval Hospital at the Naval Air Station in Sigonella, Sicily where I worked with LT Luis A. Nunez and Brenda Newlon. This was their first AABB assessment of Transfusion Service activities. Our opening meeting, or briefing, started with everyone standing at attention and saluting the Commander. A different experience for me, as I had never been in a military setting before.

This Transfusion Service was located in a small, community type hospital like many of the hospitals in Wisconsin. Many of the patients are family members of the naval staff. They have a busy obstetrics and pediatric practice. Military personnel and their families receive an extensive health exam. If anyone in the family has a significant health issue they are not assigned to Sigonella. If there were injuries or wounded, individuals would be stabilized, and moved either to Germany or back to the U.S.

The one thing that set this lab apart from any other I had ever seen was the frozen blood depot. They had twenty –80°C freezers holding 4,000 units of frozen red blood cells! As a point of comparison, at The Blood Center of Southeastern Wisconsin, there are two freezers holding about 350 frozen rare units. Much of
the blood is collected at Great Lakes Naval Base and shipped to Sicily for storage. They were equipped to be able to thaw and wash 1,730 units in 10 days. In order to do this they have a “deglyc team” of 6 trained individuals. Four members of the team sat in their small break room waiting in case they were needed when major hostilities were taking place during the 2003 war in Iraq. Another interesting difference between the military blood bank and civilian blood banks is the computer system. The Defense Blood System (D.B.S) is used by all arms of the military. I asked if we could purchase it but you can guess what the answer was.

In conclusion, AABB is recognized internationally as the leader in transfusion medicine, and laboratories worldwide are successfully obtaining AABB accreditation. The U.S. Naval Hospital-Sigonella received their accreditation. At the recent AABB annual meeting in San Diego, the Centro di Trasfusionale e di Immunologia dei Trapianti was recognized as being the first international Immunohematology Reference Lab to become AABB accredited and to join only 54 other AABB accredited IRLs now worldwide.
Platelet Bacterial Contamination

by Donna Cochenet

Besides the forest fires, the “hot topic” at the 2003 AABB Annual Meeting in San Diego was bacterial contamination, specifically AABB standard 5.1.5.1, effective 3-1-04, which states: “The blood bank or transfusion service shall have methods to limit and detect bacterial contamination in all platelet components.” Several sessions, speakers and exhibits were devoted to efforts to limit and detect bacterial contamination of platelet products. We are fortunate to have 2 reports from the AABB meeting regarding this hot topic. The first report is from Donna Cochenet, it is a review by Roslyn Yomtovian, M.D. on “Methods for Limiting and Detecting Platelet Bacterial Contamination”. The second report is from Diane Carlson and is a practical summary of these methods for use in donor centers and transfusion services.

Platelet Bacterial Contamination

Roslyn Yomtovian, M.D. from University Hospitals of Cleveland & Case Western Reserve University presented a review entitled “Bacterial Contamination: Methods for Limiting and Detecting Platelet Bacterial Contamination”. She began by providing background information on the risk to recipient safety. She referenced the BaCon study which estimated the incidence of clinically significant bacterial contamination associated with the transfusion of platelet components to be 1 in 100,000 platelet transfusions and the incidence of death estimated to be 1 in 500,000 platelet transfusions. However, in the speaker’s experience, she found the incidence to be higher (1 in 55 transfusions) if the transfusing facility proactively looks for it using screening methods (gram stains, cultures etc...) and staff recognition of signs and symptoms of possible transfusion reactions.

Her presentation continued with strategies to reduce this risk by describing methods to limit and detect bacterial contamination. Methods to limit contaminated units included: 1.) avoidance of contaminants 2.) reduction of contaminants and 3.) inactivation or growth inhibition. Recommended ways to avoid bacterial contamination were use of a thorough and careful donor screening, a meticulous phlebotomy process, use of alternatives to green soap for the arm scrub and decrease exposure to multiple donors through increase use of single donor apheresis platelets instead of random pooled platelets. Reduction of contamination could be achieved with the use of phlebotomy diversion. This process has been shown to reduce the contamination rate from 0.14% to 0.03%. The technique involves diverting the initial blood draw after the needlestick away from the primary blood collection bag into a sampling pouch. Bacteria inactivation or growth inhibition as a means to limit contaminants has not yet been found feasible. Some options include cold storage, addition of antibiotics, photochemical and chemical decontamination.

She further explained that the focus has primarily been on detection methods prior to transfusion. The ideal detection method is dependent on establishing a level of clinically tolerable bacterial contamination of platelet units. As few as 10^2-10^3 CFU/ml of S. epidemidis have been associated with delayed fevers or positive blood cultures. Ideally, methods should be rapid, sensitive, specific, inexpensive, practical and simple. The less sensitive the method, the closer the testing should be performed to the transfusion event. The various detection methods are based on differing techniques such as: 1.) measurement of metabolic products (dipstick pH &/or glucose) with a sensitivity of 10^7, 2.) cell growth indicators (cultures and % oxygen) with sensitivities of < 10^2-10^3, 3.) cell markers (swirl test, Gram stain, Wright stain) with sensitivities of 10^7 for the swirl test and 10^5-10^6 for the stains and 4.) molecular biology (PCR) with as yet unpublished sensitivity statistics.

Two strategies exist when choosing a detection method, testing near collection or testing upon issue. Testing near collection predominantly would use the culture methodology and is more suited to the blood centers or blood collecting facilities. The pros and cons of using this strategy would be: requires a large sample, false negatives, false positives, holding products for completion of testing resulting in delay of platelet availability and positive culture results may occur after the unit has been transfused. Testing the platelet unit when a request for transfusion is issued or “point of care” methodology would include the dipstick, staining or swirling techniques and is more practical for the hospital based transfusion service.
These methods use smaller sample size but the possibility for false negatives still exists. This “point of care” testing reflects the bacterial status of the unit at the time of issuance yet would delay transfusion while testing is being done.

Future developments are anticipated. Under consideration are: FDA approval of prestorage pooling of random platelet units, FDA approval to extend the expiration to 7 days, the use of detection techniques (PCR, lateral flow immunoassays) with increased sensitivity and development of bacterial decontamination or growth retarding technologies.

In summary, the presentation concluded by stressing three factors to best prevent transfusion of bacterially contaminated platelet units.

*Minimize bacterial load with meticulous phlebotomy process and preferential use of apheresis platelets.

*Reduce quantity of bacteria entering the primary collection bag by diverting the initial volume of blood drawn.

*Use of sensitive detection methods.

For further details the attendees were directed to AABB’s Association Bulletin #03-12 Further Guidance on Methods to Detect Bacterial Contamination of Platelet Components.

Bacterial Detection in Platelet Products
by Diane Carlson

Bacterial contamination of platelets is the most common transfusion associated risk at 1 in 1,000 to 1 in 3,000 platelet units. A combination of changes can be made to reduce this risk.

Blood centers can make changes which can minimize the risks associated with contamination at the time of blood collection. The amount of training and supervision of phlebotomy staff can be increased. The arm scrub procedure can be changed to an alternative, more effective solution such as chlorhexidine. Blood collection bags with diversion pouches can be used which will divert the first mls of blood collected to be used as testing samples. This will decrease the risk of the most common type of contaminants from the skin such as Staphylococcus epidermidis. Blood centers can also increase their production of apheresis platelet products, these are thought to be at less risk since the product is collected from one donor and one venipuncture versus pooled random platelets. This will require time to complete since equipment updates, additional staff, and a larger donor pool would be needed. Even accurate, thorough screening of a blood donor can not always identify an asymptomatic bacteremic donor. That is why additional testing needs to be done.

The next step to ensure a safer platelet product is bacterial detection. This can be accomplished by any or a combination of the following methods. Bacterial detection by automated systems will be used mostly to detect aerobic bacteria in apheresis products. Two systems available are the BacT/ALERT by BioMerieux and the Pall BDS. Both require holding the product for >24 hrs after collection before sampling to allow for any bacteria present to grow to detectable levels. A sterile connecting device is used to attach a sampling container. The BacT/ALERT monitors CO₂ production while the BDS reads oxygen levels. The use of either system raises questions such as when to take the inoculating sample, how long to monitor the product- until release, until positive, or until expiration and how to phrase testing results upon product release.
Educational Committee Up & Coming Events

WABB Spring Wet Workshop
The New Millennium
Rotating Tech

Location: The American Red Cross, Madison Wisconsin

Dates: Thursday - May 20 & Friday - May 21, 2004

The New Millennium Rotating Tech Objectives:

1. Using the proper technique, perform an ABO/Rh, Gel antibody screen, DAT, elution and panel.
2. Recognize and recommend solutions to serologic problems that need further investigation.
3. Discuss clinical situations in which a positive direct antiglobin test may be encountered.
4. Perform, analyze and interpret antibody panels using the “crossing out” technique.

2003 AABB Annual Meeting

Swirling is another option and intended to be used in an emergency situation or in combination with other methods. Platelets with normal metabolic functions are discoid in shape in plasma and will align their axes with the flow. When light shines through the bag they will “shimmer” and this produces a swirling effect when the bag is rotated. This is subjective and may be difficult to train staff properly.

Performing a gram or other staining method is another simple and inexpensive method to detect bacteria. These methods take some time at issue of product and may cause delays in product release and have been known to lead to false positive results and loss of products.

Urine dipsticks are an inexpensive and relatively quick method to detect bacterial contamination and is thought to be the best solution for testing random platelets. The platelet is produced with a long “tail”. The product in the tail is stripped back into the main bag 2-3 times and a segment to be tested is sealed and removed. The segment is cut open and drops of the platelet product are placed on the glucose and pH portions of the dipstick and read according to manufacturer’s directions. When bacteria are present they consume glucose and produce acids. The consensus is product acceptability when both the glucose is >250 mg/dl and pH is >7.0. Glucose and pH can also be measured by automated equipment. These measurements should be taken at the time of product release for transfusion and must be performed on each individual platelet prior to pooling. This method has also yielded false positive results and resulted in lost products.

Verax Biomedical is developing a product for bacterial detection which is simple to use, requires a 300ul sample, and can be read in 20 minutes. This product is in the process of obtaining FDA approval and is not yet available.

All of these changes and methods are an attempt to provide a safer platelet product for the patient. All will require coordination and cooperation between the blood supplier and the transfusion services, to not only meet the standard, but to achieve the intent of the new standard-to provide a safer platelet product.

Blood Bank Update

Blood Bank Update is published bi-annually. Your input is welcome.

Please submit your articles and ideas to Judy Snow, MT ASCP.

Blood Bank Update is designed by the Document and Graphic Services Department of The Blood Center of Southeastern Wisconsin, Inc., Milwaukee, WI.

“The Real ABO” continued from Page 2

Incubations were done at 37C and 4C. Following the adsorption, an eluate was prepared from the patient’s cells using a Freeze/Thaw (Lui) elution technique. Anti-B was detected in the patient’s eluate indicating anti-B bound to weak B antigen during the adsorption. These results indicate that the patient’s red cells are a subgroup of B.

This case demonstrates the following key points. First it is important to take the reverse typing gradually down in temperature. If the reverse typing in this case had been immediately taken to 4C without any controls, the patient could have been called Group O. Taking the cold panel down gradually in temperature helped to identify an interfering cold alloantibody. Secondly, it is important to run more than one example of selected cells when working up any discrepancy. In this case, all group B reverse cells were P1+ thereby giving false positive results at colder temperatures that could have been interpreted as a cold autoantibody (-I or –IH). The incidence of P1 is 80% of the population so most reverse typing cells will be P1+. Lastly, when working up an ABO discrepancy, it is imperative to include the appropriate controls, auto control and screening cells, when lowering the incubation temperature.
The WABB would like to thank the following companies for their monetary support of the Fall Seminar. For more information on any of our generous sponsors, please contact the representative listed below.

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