REPORT

2008 Immunohematology Reference Laboratory Conference

Summary of presentations

For more than 20 years, the AABB and the American Red Cross (ARC) have hosted the Immunohematology Reference Laboratory (IRL) Conference in mid-spring. Initially the conference was jointly hosted by the two organizations; it has since been hosted alternately with each organization choosing the host city and planning the conference itinerary. The conference has been held in various cities throughout the United States, including Atlanta, Chicago, Las Vegas, New Orleans, Memphis, Orlando, San Diego, and this year, Scottsdale, Arizona.

The conference begins on Friday afternoon with proctor-led case studies discussing advanced immunohematologic investigations, followed by a welcome reception that promotes connecting and networking with fellow technologists and physicians from around the country and, typically, Canada. Saturday begins with breakfast followed by speaker presentations on various serologic, technical, clinical, administrative, quality, and regulatory issues that affect today's reference laboratories. These presentations extend until late afternoon with scheduled breaks and a provided lunch. The presentations continue on Sunday with the conference ending at noon. Attendees are encouraged to bring posters for viewing, and those who do so have the opportunity to present the information to all.

Following are summaries of the presentations given at the 2008 IRL conference that was hosted by the ARC from April 11 to 13 in Scottsdale, Arizona.

Leadership

Many individuals believe that to lead you must be at the very top of an organization, but nothing could be further from the truth. In successful organizations, much of the leadership comes from below the top; in fact, most comes from the middle of the organization. Each of us can be a good leader, but we must work to obtain the skills that good leaders possess. Leadership is all about influence, getting people to follow you not because of your position but because of who you are and what you do. John Maxwell, a renowned author on leadership principles, states "The true measure of leadership is influence, nothing more, nothing less." Influence is defined as a power affecting a person, thing, or course of events, especially one that operates without any direct or apparent effort. Influence is not something that is tangible, but the ability to influence is something you have to earn and you can learn how to do it.

One can have influence by position, but the leadership that results from this is very limited. People follow you because they have to follow you, not necessarily because they want to follow you. Leadership by position doesn't get one very far; it is limited by job description, and although you do have influence, that influence is limited. The ideal state is to be able to lead individuals because they want you to lead them; they allow you to influence them. They do this because they respect you or admire you and know you care about them as people. They see you as a person who gets things done; they like what you are doing. They want to be on your team!

To lead, one must prepare. A favorite quote comes from John Wooden. "When opportunity comes, it is too late to prepare." How true this is! We must be ready to lead whenever and wherever the opportunity presents itself. So how do we do that? First of all, as John Maxwell discusses in his book *The 360° Leader,* we must learn to manage ourselves. Sometimes this is one of the most challenging aspects of being a leader. A good leader knows when to show emotions and when to hold them back. At times holding back one's feelings can be best for others; at other times a little show of emotion from you is just what is needed by your team. Knowing when and where to let your emotions out is important. For example, you may be angry during a

meeting because you are not getting the cooperation you need from some of those at the table. Letting out that anger inappropriately (i.e., pounding your fist on the table or throwing something) will only make things worse and lessen you in the eyes of those you lead. Handling the situation in a calm, rational, and professional manner will elevate you with others. They want to be like you; you have more influence over them.

Leaders must also prioritize. All of us have many things on our plate, and if we try to do everything, we become ineffective, especially as leaders. Things slip through the cracks and we constantly play catch up. Learning to say "no" is difficult to do, but the good leader is able to do this to benefit the organization as a whole. A good leader delegates and allows others to do the work, providing guidance as needed without micro-managing.

Another area of leadership that requires self-management is thinking. It is true that if you think bad thoughts, bad things can happen. If you think someone is less than a "10," you may not give them a chance to show you they are a "10." If you think you can't succeed, you won't. As a leader, you must also manage what you say. Thinking before you speak is always a good practice, and making sure that what you say has value is another. Have you ever been in a meeting with someone who always has to say something even when they have nothing valuable to say? It is frustrating for everyone. If people are going to spend their time listening to you, what you say has to have value; otherwise, you are wasting their time. Remember sometimes saying nothing at all is best, especially if you have nothing valuable to say.

Lastly, and I believe most importantly, a good leader manages his or her personal life. Making time for family and friends is critical, and doing things to get away from the everyday drudgery of work is as well. Good leaders have hobbies and outside interests; for example, they watch or participate in sports, they read, and they travel (not work travel, of course). Although it isn't always possible, good leaders also take care of physical needs such as eating well, getting plenty of rest, and exercising. Failing to take care of those physical needs can make one an ineffective leader very quickly.

So what are the characteristics of an effective leader? Leaders are secure and do not feel threatened by others. They have vision and see the big picture; their scope is outside of what is good for them and them alone. They unselfishly look at what is good for those whom they lead: the department, the organization, or beyond. Real leaders are not chameleons; they do not flip-flop on decisions, saying one thing to one person or group because that is what they want to hear and something different to another person or group because that is what they want to hear. They make decisions and use a good decision-making process. They know that failing to make a decision is a decision and often a bad one.

Leaders delight when those who follow them succeed. They are not jealous that someone else is getting some limelight; they are happy to share the kudos. Leaders see everyone as a "10" and allow them a chance to be that "10" by placing them in their strength zones. Leaders truly care about the individuals they lead. They see them as people with lives. Investing time in getting to know those you lead not only helps you to understand them better but it also builds trust. This trust allows you to gain influence with them, and they are more willing to follow you. A good leader invests power in others, setting reasonable goals and supporting individuals when needed but not totally controlling them. Leaders praise accomplishments publicly and constructively criticize privately behind closed doors. Good leaders are loyal to those who follow them. Additional good leadership practices include the following:

- Being prepared—Leaders do their homework. They understand what the desired results are, how they are trying to achieve those results, and what the issues in achieving those results are.
- Communicating well—Leaders communicate horizontally and vertically. They communicate clearly and directly. They listen well and they inform others appropriately as needed.
- Setting good goals—Leaders set goals that are realistic but they push the envelope, challenging within limits those who follow them. They don't ask others to do what they would not do.
- Modeling the behavior you want to see in others (i.e., walking the talk)—Leaders are role models and exhibit those characteristics they want to see in those who follow them. They never just give "lip service."
- Using data and facts to make decisions—Whereas sometimes good leaders go with intuition, whenever possible, they make decisions based on good data and facts.
- Managing conflict instead of avoiding it—Leaders are not afraid of conflict. Although they don't seek to create it, when it occurs they facilitate its resolution.

As you can see, leadership is much more than management. Management is about projects; leadership is about people. Management is about procedures and rules; leadership is about vision and relationships. The leader looks to the future; the manager maintains and implements what is the current state.

Not everyone is born a leader and not everyone can be at the top of the organization, but everyone can lead. Working on those areas discussed above will prepare you to lead whenever and wherever the opportunity arises.

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Decision-Making When All RBCs Tested Are Incompatible

When all, or most, RBCs react during compatibility testing, there are several causes to be considered, and questions to be answered, depending on whether the RBCs in question are from donor units, reagent RBCs, or both:

1. Autoantibody or alloantibody, or both?
2. Alloantibody to high-prevalence antigen?
3. Mixture of several alloantibodies?
4. Not an antibody to RBC antigens?
5. Is antibody clinically significant?

Items 1, 2, and 3 are the "bread and butter" of IRLs, so will not be discussed in great detail. Questions 4 and 5 are sometimes more difficult for even a sophisticated IRL to answer.

When all tested reagent RBCs react, sometimes it is attributable to antibodies that are reacting with antigens that are not blood group antigens. These antigens may be chemicals (e.g., antibiotics, sugars, EDTA, citrate, hydrocortisone, inosine) in commercial reagents such as RBC suspending media, antisera (e.g., dyes), or potentiators (Paraben, azide, thimerosal). This becomes obvious sometimes when reagents from other companies and donor RBCs do not react. Other targets for non–blood group antigens may be the senescent cell antigen present on older RBCs. An unusual phenomenon has been described in which serum reacts with all RBCs but plasma does not, and the DAT is positive on RBCs from a clot but not an EDTA sample. This condition is associated with ulcerative colitis. It has been suggested that it is caused by antibody to serine proteases (e.g., produced during clotting).

Several approaches have been used to predict the clinical significance of an antibody (e.g., thermal amplitude, specificity, functional cellular assays, and 51Cr RBC survival). A major problem is defining what clinical significance means. Do we want tests to predict whether transfused RBCs will survive normally, or are we satisfied if the antibody does not cause morbidity in the patient? It may be that the first is better for patients with hematologic disease, but the second is acceptable for most other patients.

References

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Compliance to Quality: A Journey

We are a focused industry, focused on compliance, and maybe sometimes so focused on compliance that we let quality slide. Now, there is a controversial statement. In a world in which the Food and Drug Administration rules and we have the AABB Standards to abide by, how can such a statement be made? Many of us look at compliance and quality as totally interchangeable, but in reality they are not. To explore how we might want to change our focus from one bent on compliance to one bent on quality, let's talk about a journey—a journey from compliance to quality. By the way, for those of you who are gasping for air about now, I am not saying *away* from compliance but rather from compliance focused to quality focused. This doesn't mean you are going to stop being compliant—quite the opposite. In a true quality organization, compliance is a byproduct in all you do.

First of all, let's talk about the differences between compliance and quality. Compliance by definition means bending to the will of others; it is the act of submitting. A total focus on compliance stifles creativity, is reactive, and implies something is done to you. It can,

and often does, create adverse relationships with those who regulate us. Quality, on the other hand, leads to compliance, generates new ideas, is proactive, and promotes good relationships with regulators. Quality is more than just compliance. So what is quality?

Quality is a matter of perception—McDonald's versus Chez Jardin. Which restaurant represents quality depends on the requirements. Do you want a fast-food meal that is consistently the same or a gourmet meal in a quiet, candlelit dining room?

A good definition for quality is *doing the right things right the first time.* If we know what the requirements are, we do what we should do; we are compliant. If we do things right the first time, we are not only effective but we are also efficient. Quality is the result of effectiveness and efficiency. You do what you say you are going to do and you do it right with the least expenditure of resources. A quality organization is by definition also a compliant organization because you are doing the right things right. You also please your customers because you know what their requirements are and you work to meet those requirements each time you do your work.

So, how do we achieve a quality focus? First of all there are some important questions to ask. Does what you are doing make sense? Is it the right thing to do? Is it the right thing to do *now?* Is there an easier way to do it (simpler is better)? Does the fear of making a mistake paralyze you? Does change overwhelm you? What are your customers' requirements? Knowing the answers to these questions and dealing with the difficult ones before you begin the journey toward a quality focus is critical to your success.

There are also some cautions you should remember along the way:

(1) Don't head down the wrong path by implementing complex systems that hinder you instead of helping you.
(2) Don't yield to the temptation to tackle everything at once.
(3) Know your capabilities.
(4) Be accountable.

Accountability, according to *The OZ Principle,* a book by Roger Connors, Tom Smith, and Craig Hickman, is the process of seeing, owning, solving, and doing. It is a perspective that embraces both current and future efforts rather than reactive and historical expectations.

Considering accountability, you will find some dangerous detours in your journey to quality. Let's take a brief look at these.

Number 1—Ignoring problems or denying that you have problems

People pretend not to know that there is a problem, remain unaware that the problem affects them, or choose to altogether deny the problem. This just doesn't work. You can't fix problems unless you pay attention to them. This is sort of like sticking your head in the sand. You eventually are going to have to breathe, and the problems won't have gone away.

Number 2—It is not MY job!

In this case, there is an awareness that something needs to be done to get the results, but there is also a lack of responsibility or desire to involve oneself. Assuming someone else will always pick up the ball is a mistake; things fall through the cracks or, even worse, nothing is done.

Number 3—Pointing fingers

People deny their own responsibility for poor results and seek to shift the blame to others. This is a total lack of accountability; someone else is always responsible for the state of things. This is ignoring your role in the situation, and usually you do have some role in why things are as they are.

Number 4—Excuses

Making up excuses doesn't get things fixed and may even lead to solving the wrong problem.

Number 5—Confusion—Tell me what to do

People cite confusion as an excuse to avoid accountability. If they don't understand the problem or the situation, surely they can't be expected to do anything about it. Or if you tell me what to do, then I can't be held accountable for what happens.

Number 6—Cover your tail

This happens when people seek "protection" by developing "stories" as to why they couldn't possibly be blamed for something that might go wrong. Individuals can be quite creative in making up these stories; again, they hide the truth and avoid true accountability.

Number 7—Wait and see

In this dangerous detour, people choose to wait and see whether things will get better. Typically, things only get worse. Solutions get swallowed up in a swamp of inaction. This type of behavior may be motivated by fear of failure, risk aversion, or an unrealistic desire for a better solution.

The road to quality is not an easy road; avoiding those detours can be hard. However, the road to quality is a rewarding one. A key factor is to celebrate successes (especially small ones), catch people doing the right things right, create heroes, and recognize and reward performance. Achieving quality is going beyond compliance, and it allows you to meet your goals and results in a strong organization. In a true quality organization, the compliance is there. Lastly, remember, quality is a journey and one which is truly worth making.

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You Have Options . . . Immucor, Inc.

As the need for accurate, timely, and cost-effective immunohematology diagnostic testing increases dramatically across the world, Immucor stands as the largest and best equipped corporation in North America to provide the solutions needed to meet the needs of the industry. Based in Norcross, Georgia, Immucor believes in exceeding the needs of the customer. This starts by understanding the requirements of the industry through "voice of the customer" research. In 2007, this extensive market research identified five key market needs in our industry. Based on these findings, we concluded that the blood bank customer most desires the following:

1. Full and reliable blood bank automation
2. A broad testing menu
3. A continuous access platform
4. A fast turnaround time
5. Automation and test methods that are easy to use

Immucor is meeting these needs through the delivery of superior products in our Scalable Solutions automated product line, which includes the Capture workstation, the Galileo, and the Galileo Echo.

Immucor's manual Capture workstation features the P2 dual-plate incubator, the CSW100 plate washer, and the Immuspin centrifuge. Perfect for smaller laboratories or as a backup to large, fully automated laboratories, the Capture workstation provides standardized test results every time. For more than 20 years, thousands of laboratories across the globe have trusted Capture solid-phase technology, with more than 30 million Capture tests performed annually.

Galileo, Immucor's flagship instrument, has revolutionized transfusion diagnostics with its speed and flexibility. Galileo was the first automated system designed to meet the workflow of a transfusion diagnostics laboratory, not change it. With a broad test menu, high throughput coupled with a quick turnaround time, reflex testing, and an intuitive user interface, Galileo has become the gold standard in automated platforms.

With high demand for the features of the Galileo in a smaller package, Immucor developed the Galileo Echo—a revolutionary instrument that delivers the benefits of Galileo for smaller laboratories. Echo was designed to perform a record number of assays with the industry's smallest footprint.

However, meeting the customer's needs requires not only superior products, but also a superior approach. Anchored by our Blood Bank System Specialists, the Immucor team is dedicated to providing a solution for these key industry needs, or "drivers." This includes coupling blood bank reagent options and instrument platforms with an implementation process called "Lean."

Lean is a continuous improvement philosophy that is used to eliminate waste and variation. It is widely used today in the health-care diagnostics industry to improve quality and response times while maintaining or decreasing expenditures. Immucor has invested in our people to make them Lean-certified under the Lean HealthCare Institute banner, with the express purpose to pass on their skills to Immucor customers by installing and integrating all products into the customer's specific environment appropriately.

This results in a customized solution for each laboratory location. This customized solution may include layout and process enhancements through the Lean methodology. It might also include technology enhancements through Immucor's networked automation. Immucor, through a partnership with Data Innovations, uses a variety of networking methods, including remote access and wide area networked innovations, to meet the data needs of the customer, ensuring that they have the right information at the right time.

Through dedication and the drive to exceed expectations, Immucor is committed to meet and exceed the needs of the customer.

Theresa Heflin, VP Marketing, Immucor, Inc., Norcross, GA.

The Journey to Licensure

The Diagnostic Manufacturing Division (DMD) of the American Red Cross (ARC) fully manufactures licensed and 510(k) cleared immunohematology

reagents in accordance with current Good Manufacturing Practices and 21CFR, parts 600 and 800. These products are distributed to ARC National Testing Laboratories and Immunohematology Reference Laboratories and various non–ARC organizations. As a constituent of the ARC, the mission of the DMD is to support its customers with the highest quality, cost-effective reagents and ancillary products. To that end, DMD uses a quality system that includes change management, document control, training, supplier evaluation, incoming goods specifications and inspection, in-process controls, contamination controls, environmental monitoring, labeling and packaging controls, final product testing, design control, equipment and process validation, equipment maintenance and calibration, deviation management, corrective and preventive action plans, nonconforming product control, product complaint management, internal audits, and management review.

Established in 1976, DMD has manufactured from four locations. The 1998 move to the current location required DMD to once again obtain licensure to manufacture licensed blood grouping reagents through the Biologics License Application (BLA) process.

A 2001 BLA supplement was withdrawn primarily because of an inability to meet FDA sterility requirements. A different approach using a microbiologically controlled process was developed, and a manufacturing process was designed to ensure the microbial level will not adversely impact product performance. The philosophy is based on two distinct elements and the results of supportive studies:

Environmental Monitoring
- Air
- Surfaces
- Water
- Personnel (gowning)

Bioburden Reduction
- Product filtration
- Process equipment cleaning
- Heat treatment of product components (containers/closures)
- Product preservative (sodium azide, 0.1%)

Supportive Validation Studies
- Bioburden monitoring of in-process material
- Airborne baseline levels
- Sodium azide effectiveness
- Equipment cleaning
- Closure integrity of filtration vessel
- Closure integrity of final product containers
- Media fill—tryptic soy broth substituted for product

Final product contamination testing demonstrates the product meets the DMD microbiologically controlled standard.

A BLA supplement was submitted in 2005 that contained the following required elements:

- Cover letter
- FDA forms (2)
- Introduction—executive summary
- Proposed labeling (all labels and package inserts) and packaging
- Chemistry, manufacturing, and controls (CMC)
  - Extensive description of in vitro substance (active ingredient) and in vitro product
    - Composition—ingredients and formulas
    - Characterization—test method descriptions and applications
    - Method of manufacture—raw materials and acceptance criteria, flow charts
    - Process controls—in-process controls, process validation, bioburden assessment
    - Reference standards
    - Specifications and analytical methods
    - Container closure system—description and validation
    - Stability data
  - Environmental monitoring data
    - Air
    - Water
    - Surfaces
    - People
    - In-process bioburden
  - Batch records—6 conformance lots
  - Validation (process and equipment) summaries
- Establishment description
  - Physical description, floor plans
  - Cross-contamination controls
  - Environmental monitoring
  - HVAC system and validation
  - Water system and validation

The DMD submission totaled 1315 pages that thoroughly explained the microbiologic control "story," supplied data to support the "story," and provided details about the product composition, manufacturing process, test methods, and conformance lot production.

DMD was granted licensure in 2006 for anti-Fy^a and anti-K and in 2007 for anti-S and anti-k. Another BLA supplement will occur in 2008 for anti-M, anti-s, anti-Kp^a and anti-Kp^b.

Elizabeth Cummings, Diagnostic Manufacturing Division, American Red Cross, Rockland, MD.

Serologic Investigation of Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia can be classified as those anemias associated with (1) antibodies reacting optimally at 37°C ("warm type" AIHA [WAIHA] = 80%), and (2) antibodies reacting optimally below 10°C (cold agglutinin syndrome [CAS] = 18%, and paroxysmal cold hemoglobinuria [PCH] = 2%).

- WAIHA: Usually IgG autoantibody, but can be IgM or IgA. DAT: IgG + C3 (67%), IgG without C3 (20%), C3 without IgG (13%). In vitro lysis of RBCs is uncommon (0.8% untreated, 13% enzyme-treated); 35 percent will have cold agglutinins active at room temperature. Usually extravascular lysis. Most common specificity is Rh-related, but almost all high-frequency antigens have been involved.
- CAS: IgM high titer/thermal amplitude (≥30°C); cold agglutinin. Cold monophasic lysin usually present. DAT: C3dg only. Extravascular in vivo lysis. Usually anti-I.
- PCH: Rarest type of AIHA (<2%). More common in children (infection associated) than adults. Usually intravascular lysis. DAT: C3dg only. Biphasic (sensitized in cold, hemolysis when moved to 37°C) cold lysin detected by Donath Landsteiner (DL) test. DL antibody has anti-P specificity.
- DAT-Negative AIHA: Approximately 10 percent of WAIHA. Can be caused by RBC-bound IgG below DAT sensitivity threshold, low-affinity autoantibody, IgA or IgM warm antibody. Helpful tests are direct Polybrene test, DAT using anti-IgA/IgM, using ice-cold saline or LISS washes for DAT, or flow cytometric DAT.
- WAIHA Associated With IgM Autoantibodies: Often, severe AIHA, sometimes intravascular lysis. Spontaneous agglutination of patients' RBCs often (78%) occurs; C3dg on RBCs (90%); IgM on RBCs (62%) but not often (30%) detected by DAT. Sometimes (25%) IgG also present. Serum usually (80%) contains 37°C-reactive agglutinins.

Childhood AIHA:

- Acute transient: Acute transient AIHA 10× more common in children than adults; 82 percent in first 4 years; 68 percent associated with infection; 45 percent have hemoglobinuria (71% of sudden onset); 59 percent have only C3dg on RBCs; 12 percent have positive DL test. Respond well to steroids. Low fatality rate.
- Chronic WAIHA: Often (58%) associated with systemic disorders; only 44 percent in first 4 years; 85 percent have IgG, with or without C3, on RBCs. Variable response to steroids; 12 percent mortality.

Reference

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Controversies in Testing

Although the practice of blood banking and transfusion medicine may, at times, seem to be driven by regulators, there still remain many opportunities for variation in applying the regulations to actual daily operations. Adaptation of new technologies also affords the possibility of different applications. Controversies over what acceptable or best practices may look like arise. Several of these were more closely examined.

Criteria for Exclusion of Alloantibodies

A critical control point in identification of RBC antibodies is antibody exclusion. This ensures that there are no other unexpected antibodies in the sample that may have been masked by the reactivity of the known antibodies. The process of antibody exclusion requires decisions on what alloantibodies will routinely be investigated and excluded and what criteria will be used for exclusion. The following options exist for exclusion: (1) using RBCs that carry a single dose or presumed double dose of the antigen in question; (2) using only in-date reagent RBCs or allowing the use of expired reagent RBCs; or (3) excluding an antibody specificity on the basis of one nonreactive antigen-positive RBC sample or requiring two or more antigen-positive RBC samples to be nonreactive. Decisions on these options constitute the policy for antibody exclusion in a given facility.

The biggest controversy in antigen exclusion centers on the number of antigen-positive RBC samples that must be nonreactive to exclude the antibody. Use of one RBC sample for exclusion allows antibodies to be excluded more easily with minimal sample. It also can affect the selection of units for transfusion because when an antibody is excluded, donor units negative for that antigen are not required for transfusion. However, reliance on a single RBC sample for exclusion does not allow for any error in testing or unknown variation in antigen expression. A policy that requires nonreactivity with two or more antigen-positive RBC samples to exclude an antibody compensates for these unexpected events. However, this practice can require a greater inventory of test RBCs and will likely use more patient sample.

An e-mail survey of AABB-accredited Immunohematology Reference Laboratories (IRL) and American Red Cross (ARC) IRLs was conducted in mid-2007 to assess policies related to the number of exclusion RBCs required. Of 63 laboratories responding, 42 facilities (67%) used only one nonreactive RBC sample to exclude an alloantibody. Comments from the responding laboratories were "require one example of double-dose RBCs but more than one example if the RBCs express a single dose of the antigen," or "one double dose required except for anti-K and anti-C or anti-E in presence of anti-D." Several laboratories required more than one antigen-negative RBC sample for clinically significant alloantibodies but only one example for antibodies not generally clinically significant.

Considering the pros and cons of various exclusion issues, one protocol that might be used would be as follows:

- For Rh, Fy, Jk, Ss antibodies
  - At least two antigen-positive RBC samples are nonreactive
  - At least one example of the RBCs has a double dose expression of the antigen except when excluding anti-C or anti-E in the presence of anti-D or excluding anti-K
  - If the exclusion RBC sample does not have a double dose of the antigen, the test method used must be PEG, gel, solid-phase, or enzyme (if appropriate)
  - If expired RBCs must be used, they must be double dose
- For MN, Lewis, P1 antibodies
  - One RBC sample for exclusion
  - Double dose, if appropriate

Reactivity in Various Test Methods

Many test methods are available for use in blood bank testing today: tube techniques (LISS or PEG) and non–tube techniques (automated and manual gel or automated and manual solid phase). In general, comparative studies have shown that non-tube methods are more sensitive than tube methods in detecting clinically significant alloantibodies. However, non-tube methods can also be less specific than tube methods. That is, when reactivity is detected in a tube method, it is more likely to be an alloantibody.

Most participants in the ARC IRL Conference represented laboratories receiving the majority of their samples from other blood bank laboratories. These referral laboratories primarily used tube techniques for routine testing; some could also perform gel testing. A very few could perform solid-phase tests. The hospital laboratories represented reported using both tube and gel methods. Virtually all hospital laboratories used the same method for initial antibody identification as antibody detection, although a second method would be used when the primary method does not indicate clear specificity.

Various scenarios were posed giving options for testing when nonspecific reactivity or panagglutination was observed in initial non-tube identification tests. Conference participants often indicated that tube test methods (LISS or PEG) would be used when an apparent autoantibody was detected in non-tube tests. Sporadic reactivity in non-tube tests would most often be approached by performing antibody exclusions in the non-tube test followed by selection of donor units based on a compatible crossmatch. When initial non-tube tests that react with many cells on a panel are encountered, some of the laboratories will revert to non-tube methods to look for specificity.

When designing antibody identification protocols combining tube and non-tube tests, it must be remembered that no single protocol is correct. The selected protocol must balance appropriate investigation of reactivity with timely provision of test results and blood products. Change in the reactivity in a patient's sample after transfusion may be an indicator for more rigorous testing by non-tube methods.

Provision of Genotype-Matched Units for WAA Patients

The availability of large scale, batch genotyping for patient and donor samples has opened new possibilities for selection of units for transfusion. Patients with warm autoantibody in the serum can require complex

adsorption studies to exclude alloantibodies. It has been suggested that genotyping for common RBC antigens could eliminate the need for evaluation of the sample using adsorptions if genotypically matched donor units were selected for transfusion.

Several concerns of both serologic and logistic nature have been raised for this type of protocol. Although readily available in the commercial setting, genotype testing must be performed in laboratories having personnel with the appropriate skill set for accurate performance, interpretation, and application of the test. A patient's genotype may not represent the RBC phenotype. Because of unrecognized mutations, a patient may appear to be antigen positive but still be capable of making the alloantibody. Logistically, the genotyping assays are not licensed for labeling of donor units with phenotype information. RBC phenotypes must be confirmed serologically. The impact on blood suppliers must also be considered. Large-scale donor phenotyping is not routinely available in donor centers. If a hospital transfusion service implements a protocol of genotype matching, the donor center must identify the matched units through labor intensive, manually performed serologic antigen typing. The blood centers must be prepared to allocate financial and personnel resources for this approach.

The protocol may first be used in patient populations such as patients with sickle cell anemia in whom genetic variation in RBC antigens is more commonly encountered and the benefits of phenotypically matched units have been demonstrated. Routine use in warm autoantibody patient populations for the pur-pose of streamlining pretransfusion testing may not be justified until genotyping in donor centers is readily available.

Controversies in testing patterns and protocols will routinely present themselves as new technologies and techniques are developed. Critical evaluation of the information and analysis of the impact on both patient care and provision of blood products for transfusion will result in wise choices that meet the needs of patients and transfusion medicine providers.

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Blood Doping in Athletes—Detection by Flow Cytometry

In sports, the term "doping" refers to the use of performance-enhancing substances or methods. Anti-doping rules have been adopted not only for the health and safety of the athlete but also to "protect the ethics underlying sports."[1] Blood doping refers to the use of methods to increase circulating hemoglobin levels. The resulting increase in the oxygen concentration of arterial blood can be an advantage for athletes both in competition and in training. Three methods athletes can use for this are (1) autologous or allogeneic blood transfusion, (2) pharmacologic products (e.g., recombinant human erythropoietin, rHuEPO) that stimulate overproduction of RBCs in the bone marrow, and (3) blood substitutes. Transfusion has been rumored to be used by athletes since the 1960s. There are anecdotal reports of its decline when rHuEPO was introduced in the late 1980s, but purportedly implementation of a test to detect rHuEPO in 2000 has driven athletes back to transfusion. Flow cytometry was first used for the detection of allogeneic transfusion in athletes at the 2004 Summer Olympics in Athens (there are currently no methods to detect autologous transfusion).

Flow cytometry is ideally suited for the detection and quantitation of mixed cell populations because each cell is analyzed individually. Subpopulations of RBCs can be detected based on their antigenic differences (via fluorochrome-labeled antibodies), and quantitative results can be obtained. Flow cytometry has been successfully used since the 1980s for the detection and quantitation of minor RBC populations in the following situations: fetal-maternal hemorrhage, survival studies in transfused patients, individuals with blood group mosaicism, and hematopoietic chimerism in twins and bone marrow/stem cell transplant patients. Recent publications have addressed the use of flow cytometry in the setting of blood doping.[2–6]

Technical Considerations

Flow cytometry is widely used for WBC analyses and less commonly for RBC analyses. Thus, most flow cytometry operators are not familiar with issues associated with testing RBCs (e.g., problems associated with agglutination); some methods that pertain to WBCs (e.g., incubations at 4°C to prevent capping) do not apply to RBCs. Agglutination is the biggest problem facing those analyzing RBCs by flow cytometry. The best flow cytometric test would involve incubation of test RBCs with a nonagglutinating, strongly fluorescent IgG blood group antibody. Unfortunately, these types of antibodies are not readily available. The usual testing protocol involves incubating test RBCs with a commercial blood group antibody (e.g., anti-c, -K, -Fy^a, etc.) and then a fluorochrome-labeled secondary

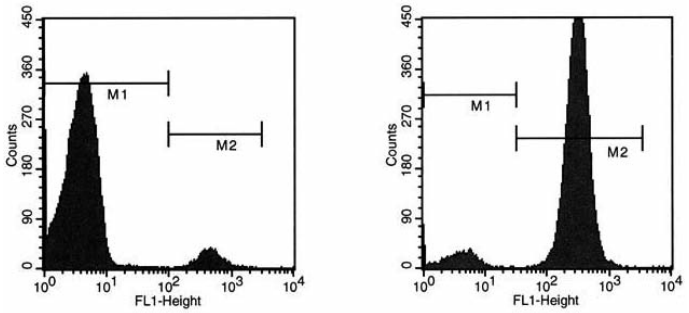

Fig. 1. Mixed RBC populations. Fluorescence histograms showing results of 5 percent s+ in s– RBCs (left histogram) and 5 percent s– in s+ RBCs (right histogram) after incubation with polyclonal anti-s and then a fluorochrome-labeled Fab anti-human IgG. Markers are set electronically around the two populations (M1 = antigen-negative events, M2 = antigen-positive events) and quantitative results are obtained.

antibody, e.g., FITC anti-IgG. Agglutination can occur due to the primary or the secondary antibody and can affect the results (the flow cytometer will count both a single RBC and an agglutinated group of RBCs as one event each). Agglutination can be avoided by using nonagglutinating primary antibodies (if available) and Fab fragments of fluorochrome-labeled secondary antibodies. In some cases, chemical fixation of RBCs may be needed to minimize agglutination. Primary and secondary antisera used for this testing will need to be standardized for use with RBCs by flow cytometry.

The RBC antigens that will be most helpful in detection of transfused RBCs in athletes are those of moderate frequency (A, B, D, C, c, E, M, N, S, s, K, Fy^a, Fy^b, Jk^a, Jk^b). To be able to clearly distinguish antigen-positive RBCs from antigen-negative RBCs (Fig. 1), it is important to use strong antibodies. Other factors that affect the ability to discriminate antigen-positive from antigen-negative RBCs include background fluorescence, the number of antigen sites, and the method used to sensitize or label the RBCs. The sensitivity for detection of an antigen-positive minor RBC population is less than 1 percent, but detection of an antigen-negative minor RBC population is more difficult (because of the presence of nonfluorescent background events).

A false-positive result would involve the detection of a second population of RBCs in an untransfused athlete. This could be attributable to poor laboratory techniques (wrong sample tested or contaminated sample) or the presence of WBCs in the sample. To have confidence in the results, mixed populations for at least two blood group antigens should be detected and the percentages of the minor populations should be similar. Antidoping test laboratories require repeat testing on a stored aliquot if the initial results are positive. Subsequent blood samples should be obtained from an athlete to demonstrate a decrease in the minor population over time. The athlete may claim that the second population is the result of chimerism rather than transfusion, but chimerism is unusual and could be detected by molecular testing.

A false-negative result would involve not detecting a second population of RBCs in a transfused athlete. It would be unlikely that there would be no antigen mismatches between the donor and recipient (unless they were identical twins), but a difference in antigens may not be detected if too few antibodies (or the wrong antibodies) are tested. Poor techniques (insufficient washing after incubation with antisera, presence of agglutination, or suboptimal instrument set-up), insufficient labeling of antigen-positive RBCs, or poor quality of the blood sample could all lead to false negative results.

In conclusion, flow cytometry is a good method to detect allogeneic transfusion. It is important that the testing laboratory be familiar with testing RBCs by routine serology as well as by flow cytometry.

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