

Tribute to John J. Moulds

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John J. Moulds, MT(ASCP)SBB



John J. Moulds had a prolific career in immunohematology. His name is synonymous with *analytic scientist*. From my perspective, John's most admirable trait and one that we should all strive for was his genuine and incisive interest in the case, whether presented by an esteemed colleague or a student. He endeavored to find the reason for the results and what it meant for the patient's care. He was a truth seeker, eagerly remembering the past to help solve today's case! One of my favorite recollections is talking at length (was there any other way with John?) about a very minute detail of a most complex case from the past, which of course was always the clue that led to the resolution. He was always the one with the corporate memory; it was John who could tell us the family members by name and type, which laboratory studied the case, the exact reactivity of the samples in each of the media, and the conclusion for the case.

Immunohematology benefitted immeasurably from his experience and ideas, and we miss his presence. John served on the editorial board of *Immunohematology* for many years, and I and the rest of the editorial board members are truly grateful for his contributions and opinions. In one of his last attendances at the annual editorial board meetings, John offered to do the centerfold. Let me explain. *Immunohematology* has a new feature that will be used, when appropriate, to help educate our staff and students. This is a page within the journal containing serologic, technical, or blood group information that can be separated and posted for educational purposes, in other words, a centerfold. Alas, John did not get to complete this. But the first centerfold, with his beloved "orphan" antigens of the 700 series, appears in this issue. The tributes in this issue give credence to the intensity of his aura in the transfusion medicine science community.

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For the 2011 South Central Association of Blood Banks (SCABB) annual meeting, I nominated John Moulds for the Larry L. Trow Memorial Education Award. This award is for people who have shown excellence in education in blood banking. The following is a reiteration of my nomination of John for the award, which, by the way, he won.

John Moulds spent his career providing education to the blood bank community in many different aspects. I first met John while I was an SBB student when he was working at Gamma Biologicals in Houston, Texas. I do believe my knowledge of the Lewis system and my philosophy of antibody identification came from that short week with John. I will never forget him saying, "Don't look for unicorns until you have ruled out the zebras."

Besides his work with Gamma, he worked for Ortho as a Senior Research Fellow and more recently as the Director of Scientific Support Services at LifeShare Blood Center. No matter what his job was, he always found time to teach at local, national, and international meetings. In 1972, he founded and was director of the Serum, Cells, and Rare Fluid (SCARF) International Exchange. John has chaired committees for AABB and SCABB; he was a peer reviewer for *Vox Sanguinis* and *Transfusion*, and served on the editorial board for *Immunohematology*.

John's association with my SBB program prompted me to nominate him for the Larry L. Trow Memorial Education Award for Education. He spent one day with my SBB students telling them how to become the best blood bankers ever, and he did this on his own time and with his own money! When answering students' questions, he would ask beforehand, "Do you want the long or the short version?" I do think his goal was to instill his love of antibodies in the future blood bankers. He had my students on the edge of their seats waiting for the next story, the next tidbit of information. He was a role model for teachers and for anyone who wanted to make antibody identification their life's work. He would pick up the phone and talk to somebody who was emailing about a problem. The email may have been a broad email that just happened to get to him, but he would take the time to actually pick up the phone and call. John taught each time he answered a question. He

had a rare gift of being able to teach with a flair that left the students in awe as they knew they were hearing words from the master!

John has won the Ivor Dunsford Memorial Award from AABB, the L. Jean Stubbins Award from SCABB, the A. Konugres Lectureship from MABB, and the Sally Frank Memorial Award from NBF/AABB, and was recently honored at the 49th SCABB meeting with the Scientific Award Lecturer. But to me the most important award is missing, and that is the Larry Trow Memorial Award for Education. John was a true educator and should be the winner of every education award possible. Education has been his true calling.

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John J. Moulds and his adventures in blood banking from the 1960s to 2011

John James Moulds started his career in immunohematology and transfusion medicine in 1964 at St. John's McNamara Hospital in Rapid City, South Dakota, as a Medical Technology (MT) student. The medical directors were Dr. Geib and Dr. Frost, and his MT instructor was Mrs. Bonnie Fingerhut. It became obvious that blood banking was John's favorite department. Here he is as a student doing blood typing (notice no gloves) (Fig. 1). There were three others in John's class—Jo Ashburn, Mary Ramos, and Irene Coates (Fig. 2). One day, John and Jo were scheduled in the blood bank, and the supervisor of that department failed to show up for work. John and Jo just carried on, typing donor and patient blood, performing antibody screenings, and crossmatching and issuing blood. Bonnie Fingerhut was rather upset when she found out they were doing all this without supervision!

John worked at St. John's for a while after graduation, and in December 1965 he took a job in a small laboratory at Chadron Community Hospital in Chadron, Nebraska, where he had attended college. He was the only technologist for several months until I was hired to help out. A variety of tests were performed, especially in the busy departments of hematology, chemistry, and blood banking. Unfortunately, there were no panels to identify antibodies, and they had to be sent out. John was almost always right on his guesses as to the

specificities. One time he helped one of the physicians diagnose a patient with lupus erythematosus from the butterfly rash on the patient's face.

John applied and was accepted to Specialist in Blood Bank (SBB) school in 1968 at War Memorial Blood Bank (now known as Memorial Blood Center) in Minneapolis, Minnesota. Here he is with the laboratory director, Eleanor Amberg, and his SBB instructor, Helen Arndt (Fig. 3). The medical director of the blood center was Dr. Herbert Polesky. John's favorite department was the reference laboratory, which was directed by Jane Swanson, one of the pioneers in the field of blood groups. She would be the one who fueled his passion for the many fascinating aspects of the rare blood groups, many of which were identified or investigated in that laboratory. This is a picture of Jane and John from 1997 when she visited him at Gamma Biologicals in Houston (Fig. 4).

After graduation from SBB school, John was a research technologist at the blood center from 1969 to 1970 until he took over Jane's position as supervisor of the reference laboratory.

The American Association of Blood Banks (AABB) rare donor file was moved from Chicago to Minneapolis in 1974, and John directed the activities until 1975. One of the first units of blood distributed internationally from Minneapolis was a group O, D-, Vel- rare unit of blood for a patient in South Africa. John was also a member of the AABB Reference and Rare Donor committee from 1972 to 1978; he chaired it from 1975 to 1978.

Early on John became involved in education when he and I were part of a team that presented American Society of Clinical Pathology (ASCP) paternity workshops. Drs. Herbert Polesky and Richard Walker gave the lectures, and John and I directed the "wet" workshops. Yes, "wet" and in hotels!! We also, of course, were the ones who prepared the samples. The workshops were held in Boston, Massachusetts, in 1971 and Atlanta, Georgia, and San Francisco, California, in 1972.

John founded the Serum, Cells, and Rare Fluids (SCARF) program in 1972. He contacted more than 60 reference laboratories, and approximately 30 responded. Each member agreed to send 10 mL of a rare or unusual sample once a year to each of the other members. John sent the first sample, which was blood from an Rh-null patient. It did not take long before the other 30 or so laboratories that had not responded wanted to join. The Rh-null sample was from Dr. Alvin Lebeck, a veterinarian in Wisconsin who John had become friends with when we went to Wisconsin on vacation to draw blood from him and his family (Fig. 5). This led to John being invited to give a talk in 1973 at the annual seminar of the AABB, which that year was called "A Seminar on Recent Advances

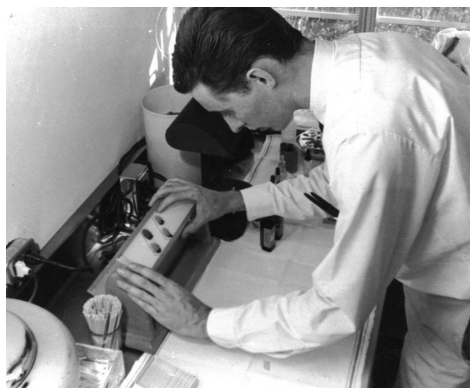


Figure 1. John Moulds as a Medical Technology student at St. John's McNamara Hospital in Rapid City, South Dakota, 1964.



Figure 2. John Moulds with his classmates in medical technology—Jo Ashburn, Mary Ramos, and Irene Coates, circa 1964.



Figure 3. John Moulds with the laboratory director, Eleanor Amberg, and his instructor, Helen Arndt, at the Specialist in Blood Bank (SBB) school at War Memorial Blood Bank (now known as Memorial Blood Center) in Minneapolis, Minnesota, circa 1968.

in Immunohematology.” This was his first major presentation. The title of the talk was “Rhnulls: amorphs and regulators.”

While at the blood center, John was fortunate to work on samples from many patients with rare blood types and antibodies. He learned a great deal from his mentor and special colleague Jane Swanson, who always said that John was her “second son.” Some of these exciting cases included Rh-nulls, D, Dombrock, Colton, Cartwright, Gregory, Hy, Knops, York, JMH, Chido, and Rodgers, to name a few. Several early publications were (1) some observations on the T, Tn, and Sd^a antigens and antibodies that define them in 1972; (2) blood group U antigen on Rh-null leukocytes in 1974; and (3) observations on the Gy^a and Hy antigens and the antibodies that define them in 1975.

John was given the opportunity to become the Director of Consultation and Education at Gamma Biologicals, Inc., in Houston, Texas. The family moved in August 1975 from cold Minnesota to hot and humid Texas. I was offered the position of Supervisor of Consultation and Education. On the first day of work there were more than 75 samples waiting to be tested. At that time, and until 2004 when the laboratory was closed, Gamma Biologicals offered a service to their customers worldwide: a no-charge workup on difficult, unusual, and rare samples. Local SBB students had been invited to spend a week at Gamma Biologicals to learn the various aspects of reagent and red blood cell (RBC) screening and panel production. Three students from St. Luke's Hospital (Carl Northam, Pamela Lacey, and Barbara Fryer) caught our eye, and shortly after the three graduated from MT school they were offered positions at Gamma Biologicals. Carl became the Supervisor of Quality Control, and Pam and Barb were hired in the Consultation and Education Department. They were the first of several technologists to work with us on samples and publish results of our findings on many interesting cases.

In this picture (Fig. 6) (left to right standing) are John Case (Director of Regulatory Affairs), Dr. Jacob Struck (Research and Development), Leah Walthers, Barbara Fryer, Mary Ann Bridges, John Moulds, and (seated) myself and Pamela Lacey. We were very fortunate to have the two gentlemen to turn to for advice on rare cases and production of special solutions. We should also recognize two administrative assistants, Barbara (Babs) Smith and Rose Quiroz.

The consultation laboratory was certified by the state of Texas and eventually became a laboratory certified by the Clinical Laboratory Improvement Act (CLIA) and also an AABB Immunohematology Reference Laboratory (IRL). Around this time SCARF expanded into two groups, which now included international members sharing samples from all over the world. John also added a third group of small reference laboratories in the United States to assist them in building up their collection of rare cells, serums, and fluids. SCARF helped us to solve many blood group problems and build up one of the largest liquid nitrogen and plasma collections of rare and unusual samples in the world. It consisted of ABO subgroups, Rh typing discrepancies, polyagglutinable cells, high- and low-incidence antigens and antibodies, and, most importantly, family studies that would be very useful for molecular studies in the future. It also helped approximately 150 laboratories throughout the world solve unusual and difficult problems. As many know, John rarely tossed an interesting sample, and to this day, LifeShare Blood Centers in Shreveport, Louisiana, under the leadership of the CEO Margaret Wallace, MT(ASCP) SBB, continues to preserve these rare samples, even though it can be very costly to maintain the rare cells in liquid nitrogen.

An official Gamma Tutorial was started for Gamma Biologicals customers in the United States that eventually

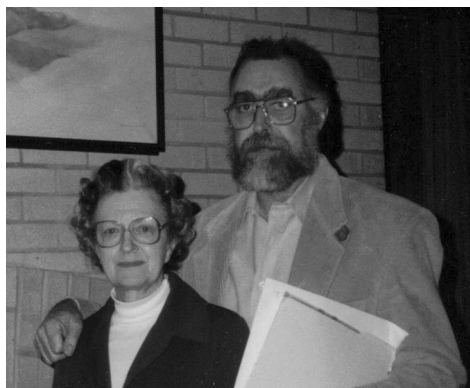


Figure 4. John Moulds and Jane Swanson at Gamma Biologicals in Houston, Texas, 1997.



Figure 5. Dr. Alvin Lebeck, a veterinarian in Wisconsin who was of the Rh_{null} phenotype, circa 1972.



Figure 6. Staff at Gamma Biologicals, Houston, Texas. Left to right standing are John Case (Director of Regulatory Affairs), Dr. Jacob Struck (Research and Development), Leah Walthers, Barbara Fryer, Mary Ann Bridges, John Moulds; seated are Marilyn Grandstaff (Moulds) and Pamela Lacey.

would lead to International Tutorial programs. It began with two invited customers spending Monday through Friday working with the consultation staff on interesting samples and learning about blood groups. In 1980, a special laboratory was built that would accommodate 12 participants (sometimes more would be added by John as he hated to turn anyone away).

The first group of classes began in 1982, and six to eight classes were held during the year. Lectures were given by the consultation staff and other departments in the morning, and a “wet” laboratory was conducted in the afternoon, in which participants worked on almost every type of unusual sample using various techniques. More than 1000 blood bankers went through the Tutorial Program until it closed in 2006. Many of these participants are leaders today in the field of immunohematology, including several physicians.

As John read publications on various techniques, he would start making reagents that were needed to perform them. He then gave them to the consultation staff to use on cases, and would then publish the results. He even had his mother, Edith Moulds, growing *Vicia graminea* seeds to make our own lectin, and these details were outlined in a poster presented at the AABB. Most of the homemade reagents eventually became commercially available from Gamma Biologicals, and ultimately other reagent and RBC manufacturing companies followed suit on some of these. This subject will be discussed by Tony Casina in this issue.

As if this was not enough to keep John busy, he also traveled nationally and internationally giving talks on blood group findings, techniques, etc. John presented his second major talk in 1978 for the AABB Preconvention Seminar in Seminar on Perinatal Blood Banking. The topic was “Immunosuppression by Passive Antibody, Rh D Suppression—Rationale and Use and Antenatal and Postnatal Prophylactic Treatment.” Rh was

probably one of his most favorite blood groups, and he was involved from the very beginning of his career with many of the new findings in this blood group system.

There were several other blood group systems John had a special interest in, one of which was Colton, in which the first Co(a–b+) was discovered in Minneapolis and a patient sample studied in Houston by John and the staff was the first Co(a–b–). He was very proud to be included in publications with Peter Agre, who received the Nobel Prize for Chemistry for his work on aquaporin 1 and Colton and also was the recipient of the Karl Landsteiner award from the AABB.

Cromer was another blood group system that John became involved with, when one of the staff worked on a sample from the second example of IFC negative, which is the null of Cromer. In collaboration with Cyril Levene in Israel our laboratory described Dr in the Cromer system, and later on John would work with Bogdan Nowicki in Houston to describe a hemagglutinin of uropathogenic *Escherichia coli* recognizing the Dr blood group antigen.

John was also very fortunate to be able to work with the German scientist Wolfgang Dahr, who had a particular interest in the MNS and Gerbich blood group systems. It was interesting to see the two of them in the laboratory preparing membranes and testing unusual human and monoclonal antibodies.

John was a member of several professional organizations (often chairing committees) and editorial boards. The organizations included AABB, ASCP, South Central Association of Blood Banks (SCABB), International Society of Blood Transfusion (ISBT), and Invitational Conference of Investigative Immunohematologists (ICII).



Figure 7. John Moulds receiving the L. Jean Stubbins Award from SCABB in 1979.



Figure 8. John Moulds with his mother, Edith Moulds, and his daughter, Terri Moulds Bowen, PhD, upon receiving the Distinguished Alumni Award from Chadron State College, 2009.



Figure 9. John Moulds with his mother, Edith Moulds, his daughter, Christine Moulds-Merritt, MD, FACS, and his grandsons, Daniel Bartley Merritt III (age 13) and Matthew Ryan Merritt (age 9), at the dedication of the LifeShare laboratories in 2011.

He was on the ISBT Working Party on Terminology for Red Cell Surface Antigens since 1980 and chaired the committee from 1990 to 1994. He also was on the 3rd and 4th International Workshop and Symposium on Monoclonal Antibodies Against Red Blood Cells and Related Proteins program committees in 1995 to 1996 and 2000 to 2001. Both of these committees helped shape the organization and naming of antigens in the human blood groups and set criteria for inclusion in a blood group system.

John received numerous honors and awards from various organizations for his contributions to the field of immunohematology, transfusion medicine, and education. The first of these was the L. Jean Stubbins Award from SCABB in 1979, which he and I shared (Fig. 7). Next were the Ivor Dunsford Memorial Award from AABB in 1983, Angelyn Konugres Lectureship from MABB in 2001, Sally Frank Award Lectureship from NBF/AABB in 2003, Kay Beattie Award Lectureship, Michigan Association of Blood Banks in 2005, L. Jean Stubbins Memorial Lectureship from University of Texas Medical Branch in Galveston, Texas, in 2007, SCABB 49th Annual Meeting Scientific Award Lecturer from SCABB/CBBS in 2007, Award for Technical Excellence from America's Blood Centers/Institute for Transfusion Medicine in 2011, and the Larry L. Trow Memorial Education Award from SCABB in 2011.

One honor that John received outside the field was recognition by Chadron State College in Chadron, Nebraska, when he was presented with the Distinguished Alumni Award in 2009 for his many accomplishments. Before the awards ceremony he gave a talk to the various classes of premedical students on how to succeed in whatever you set your sights on and reach for the stars.

While at Gamma Biologicals, John went on to become Chief Operations Officer and Executive Vice President, then President and Chief Science Officer. He helped automate various aspects of manufacturing and brought computers into the workplace. He was also a member of the board of directors from 1992 to 1997.

John left Gamma Biologicals in 1997 and worked for a year as a research associate in the Department of Medical Hematology, Baylor College of Medicine, in Houston, Texas, and from there he joined Ortho Clinical Diagnostics in Raritan, New Jersey, in various capacities in research.

John left Ortho in 2004 to become Director of Scientific Support Services at LifeShare Blood Centers in Shreveport, Louisiana. One of the first items on the agenda was to attend a workshop and read articles on monocyte monolayer assay (MMA) and develop the assay at LifeShare for assisting in determining the clinical significance of antibodies when patients needed to receive incompatible blood. Again, he led this laboratory and the staff to become a source of knowledge and expertise for the reference laboratory at LifeShare and other laboratories throughout the blood bank community. This Scientific Support Laboratory has also become one of the leaders in the field of DNA technology, led by Dr. Joann Moulds. In February 2011, LifeShare honored John with a dedication ceremony at which the reference and scientific laboratories were renamed the John J. Moulds Reference and Scientific Support Laboratories for John's lifetime accomplishments. Many colleagues attended this dedication, and others sent letters of their personal memories of John.

On a personal note, John has two daughters who went on to college and got advanced degrees (there was no question in John's mind that they would). He was very proud of them

and their accomplishments. One, Terri Moulds Bowen, PhD (Fig. 8), received her advanced degree in biology from UCLA in California and loved working in the laboratory and research (like her father). However, she has now found a new career in teaching, again like her father, who never turned down anyone who would ask him for advice or to give a talk, anywhere in the world. His other daughter, Christine Moulds-Merritt, MD, FACS (Fig.9), who completed a surgery residency at Scott and White in Temple, Texas, is a general surgeon and specializes in breast surgery.

John's two grandsons, Daniel Bartley Merritt III (age 13) and Matthew Ryan Merritt (age 9) (Fig. 9), have also inherited some of John's traits—his skill for seeing a problem and coming up with solutions and his sense of humor. They both seem to find science their favorite subject in school, so it will be interesting to see what lies ahead for them in the future. They also have gone deer hunting with their mother and father, just as John did with his dad, and the oldest grandson got his first buck last year. The younger one also shares John's love for fishing.

And lastly, John's love of animals, especially dogs (Fig. 10), has led to his rescuing them from numerous situations and giving them a home filled with kindness and love.

What will be John's legacy, you might ask? Some say it is the international SCARF program he founded, which is still active today. I say this and his love of blood groups, high- and low-incidence antigens, investigating unusual test results, preparing test solutions, his sharing of the knowledge he gathered over the years, his joy in teaching, and his sense of pride in what he did throughout his career. He always reminded us to "never forget the history." He loved the stories that went with the discoveries of the blood groups and the various antigens and antibodies.

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 John Moulds Reference Laboratory
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Figure 10. John Moulds with Bodie.



The antibodies that no one wanted to work on—except John

When preparing the titles for our talks at the laboratory dedication, one of the laypersons who organized the meeting read my title and commented, "Oh, you mean like little Orphan Annie Bodies." And indeed, the HTLA group could have just as easily acquired this name had it not been for John's persistence to investigate them. Those of you who have heard me speak know of my campaign to stomp out the term *HTLA*. However, to understand where we are today means that we have to appreciate the history behind the terminology—a history in which John Moulds was a key player.

The beginning of the term HTLA goes back to the early 1970s when the American Association of Blood Banks (AABB) and American Red Cross reference laboratories had joint meetings actually consisting of wet workshops! The participants would bring antibodies to high-incidence antigens and antibodies to low-incidence antigens and try to pair them up or place them in a blood group system. But there always seemed to be a group of antibodies that never fit into a known blood group system. These antibodies were notoriously weak and difficult to work with, hence the name *serum hemagglutinins of inscrutable type* proposed by John Judd. The resulting acronym made some serologists snicker, whereas others were offended. So finally Jane Swanson, Delores Mallory, and John Moulds came up with a descriptive name of high titer–low avidity, or HTLA, antibodies. Technologists would come to automatically use this as an explanation for weak anti-human globulin (AHG)–reactive antibodies, and others would incorrectly use the name HTLA antigens. John was to live long enough to regret that name. In fact, he once wrote that "serologists frequently use slang or colloquial statements that are intended to be descriptive of the general problem... These slang terms are not intended to define an antibody specificity, but rather to roughly describe the serological results."

So what are the serologic characteristics of the HTLA antibodies? Probably their hallmark is their weak and variable reactivity at the AHG phase of testing. The reactions can range from microscopic + to 1+ or sometimes 2+. Although they were initially believed to be of high titer (>64), not all exhibit this characteristic and often the titer is dependent on the indicator cell chosen for testing. In general, they are not enhanced with low-ionic strength saline or polyethylene glycol, and the effect of enzymes varies with the individual specificities. Cord cells,

as well as older stored red blood cells (RBCs), often give weaker or negative reactions. Finally, the antibodies have not been reported to bind complement or cause hemolytic transfusion reactions. The antibodies originally meeting these criteria included anti-JMH, -Yt^a, -Gy^a, -Hy, -Jo^a, -Ch, -Rg, -Kn^a, -Kn^b, -McC^a, -McC^b (McC^c/McC^d/McC^e), -Sl^a, -Vil, -Yk^a, and -Cs^a. Each of these has now been placed in a system recognized by the International Society of Blood Transfusion, and they are described in subsequent sections. The exception is Cs^a, which remains in a collection of two antigens.

System 011-YT

YT (Cartwright) existed as a blood group system before the terminology HTLA gained favor; however, because of the antibodies' weak reactivity, many placed them into the HTLA group. Presently there are two known antigens, Yt^a and Yt^b, and only one reported example of a true null phenotype Yt(a-b-). John's involvement with this system was at the population genetics level. Working with Dr. Cyril Levine in Israel he studied Israeli Jews and they showed that this ethnic group had a high incidence of Yt(b+), approximately 25 percent. The Yt^b antigen has also been found with increased incidence among Arabs but is almost nonexistent in Asians. Therefore, one of the best sources for Yt(a-) donors is the Jewish population.

In the early 1990s, Dr. Dave Anstee in Bristol, United Kingdom, and Dr. Marilyn Telen in Durham, North Carolina, both reported that the YT system antigens were carried on a protein known as acetylcholinesterase. Thus, paroxysmal nocturnal hemoglobinuria type III RBC, which lack all glycoposphatidylinositol-linked proteins, will have the acquired phenotype Yt(a-b-). A mutation at codon 322 changes histidine (Yt^a) to arginine (Yt^b). Using this known single nucleotide polymorphism, DNA-based methods have now been developed to accurately type for YT.

System 014-DO

John was destined to be involved with the Dombrock system because his mentor at Minneapolis War Memorial Blood Bank, Ms. Jane Swanson, was the first to report anti-Do^a. He was able to squirrel away probably the biggest stash of anti-Do^a on the continent, and he used it sparingly but wisely. Being the generous soul that he was, John shared some of this antibody with Dr. Nakajima from Japan, and they were able to show that Do^a was a low-incidence antigen in that population. Later, with Dr. Yoshida Okubo, he would report the first example of Gy(a-) with anti-Gy^a in the Japanese.

Anti-Hy (Holley) was reported in an abstract in 1967, and several more examples were published, all being found in Blacks. The consultation laboratory that John directed at Gamma Biologicals studied a number of these unique antibodies and described a subdivision that they named Jc^a. Although others thought that Jc^a was synonymous with Jo^a, John always believed that they were different, and there is some emerging molecular data that may support his theory. John would also be the first to recognize an association between Hy and Gy^a as all Hy- Blacks were Gy^a weak. Finally an explanation came forth in 1995 when Banks reported that RBCs having the rare phenotype of Gy(a-), Hy-, Jo(a-) were also Do(a-b-), i.e., Gy(a-) was the null for this blood group system. This is a rare null phenotype occurring in less than 1:10,000.

The Dombrock antigens would later be determined to be located on the adenosine 5'-diphosphate ribosyltransferase 4 protein, and the molecular mechanism for each would be identified (Table 1). John was quick to remind technologists that although most HTLA antibodies are considered clinically insignificant, anti-Do^a had caused delayed hemolytic transfusion reactions. He believed that the availability of donors molecularly typed for Do^a and Do^b would be a significant improvement in supplying safer blood for these antibody producers.

System 017-Ch/Rg

Table 1. Molecular background for the common DO antigens

Phenotype	Nucleotide	Exon	Amino Acid
Do(a+)	A	2	Asn265
Do(b+)	G	2	Asp265
Hy+	G	2	Gly108
Hy-	T	2	Val108
Jo(a+)	C	2	Thr117
Jo(a-)	T	2	Ile117

John hated using "selected cells" to determine antibody specificities. He thought that serologists should use their knowledge of the antigen biochemistry and be able to manipulate the test medium. The remaining systems exemplify this opinion. After the identification of Chido (Ch) and Rodgers (Rg) antigens on the fourth component of complement, specifically C4d, John theorized that one could enhance the antibody reactivity by increasing the amount of C4 on the reagent RBC. In fact, that's just what he did. In a report in *Transfusion*, John Judd and John Moulds showed that you could use a simple sucrose method to put more C4

on the RBC, and with the increased number of antigen sites, anti-Ch or anti-Rg could react as a direct agglutinin. This, in combination with the fact that ficin destroyed antibody reactivity, made it simple to identify anti-Ch or anti-Rg. The fact that some Ch/Rg antibodies have caused anaphylactic reactions when transfusing plasma components makes it important to be able to at least get the HTLA antibody into the correct system.

Working with Dr. Carolyn Giles, John and the staff at Gamma Biologicals helped define six Chido antigens and three Rg antigens. Later, Dr. Yung Yu would work with Carolyn to define all of these at the molecular level. The rare null for this system would be the total C4-deficient patient, of which there were fewer than 20 known in the world. This fact would lead to John's next mark on the Ch/Rg system, i.e., mentoring a struggling graduate student (Fig. 1).

Dr. Joann Moulds would go on to investigate two C4-deficient (Ch-/Rg-) brothers as well as the association of the Rg- phenotype with systemic lupus erythematosus. Using monoclonal anti-Ch and anti-Rg, assays were developed to quantify these plasma proteins. One of the surprising

observations from this work was that Blacks had higher levels of C4B in their plasma, i.e., Chido substance, which was the result of a *C4B* gene duplication. This finding validated John's earlier observations that Black donors made better C4-coated RBCs for the manufacturing of Gamma Biological's reagent quality control kit.

System 022-KN

Once again, John's Minneapolis connection would contribute greatly to his involvement in a blood group system, this time Knops. Mrs. Knops was identified as having anti-Kn^a, and one serologically compatible donor was found, i.e., Margaret Helgeson. Margaret was a technologist at the blood bank and found later that she was not only Kn(a-) but was, in fact, the serologic null for the system. She became infamous when John made her a regular contributor on the international exchange he founded known as SCARF (Serum, Cells, and Rare Fluid Exchange).

When John and Marilyn Moulds went to Gamma Biologicals, they continued to work on the weak antibodies no one cared about and began adding new specificities to the KN system. These included the Hall serum (anti-Kn^b), anti-McC^a, anti-Sl^a (Swain-Langley or Sl1), and anti-Vil (Villien or Sl2). The latter two were only presented in an AABB abstract because of a difference of opinion with another collaborator, Dr. Lyndall Molthan. Over John's objections, Dr. Molthan continued to add these new specificities to the McCoy locus, i.e., McC^c, McC^d, McC^e, etc. Molecular analysis would later prove the Gamma Biologicals group correct that these were separate mutations on the Knops protein.

As if there were not enough Mouldses involved with the HTLA story, the new Dr. Moulds would take on Knops as one of her postdoctoral projects. Armed with many of the original antisera provided by John, she found that the Knops antigens resided on complement receptor type one (CR1). The variability in reactivity as well as the Helgeson phenotype could now be explained by the known inherited RBC expression polymorphism of the *CR1* gene.

But this was just the tip of the iceberg, and John's collection of antigen-negative cells and Knops antisera would prove invaluable for the next set of investigations. Dr. Lou Miller (of Duffy blood group and malaria fame) was now studying *Plasmodium falciparum* malaria and the phenomenon known as rosetting. Because John had shown that certain Knops phenotypes such as Sl(a-) and McC(a-) occurred more frequently in Blacks, Joann postulated that this may also be a protective phenotype. So off to Africa (Mali) the two traveled



Figure 1. John the consummate mentor (for Dr. Joann Moulds and many others).

to begin what would become a 6-year project. The data are too numerous to discuss here, but the conclusions were as follows: (1) the African haplotype McC(a–b+), Sl(a–), KCAM-negative results in reduced rosetting of infected RBCs and less severe malaria; (2) in Asia the protective phenotype is the Helgeson type, which also results in reduced rosetting; and (3) the most virulent forms of *P. falciparum* use CR1 (Knops) as an alternative invasion pathway into the RBCs. So much good scientific information has come from those silly serologic studies that John championed.

System 026-JMH

Perhaps it is fitting that the last system arising out of the HTLA group would share part of John Moulds' name, i.e., JMH (John Milton Hagen). This strange collection of antibodies went by many names: The Boys, The Cats, The Over 60s, etc. This was because many of the first examples were found in elderly men or women who owned cats. The antibodies were rather unexciting, not causing hemolytic disease of the newborn or fetus or hemolytic transfusion reactions, and occasionally one would appear to be an autoantibody. But again, John would be involved in elevating this group to full system status. In 1991, he and Marilyn Telen published a paper in *Blood* locating JMH on a phosphatidylinositol-linked membrane protein. Later this was identified as semaphorin A (SEMA7A or CD108). Using samples sent to him by Dr. Cyril Levine many years earlier, John collaborated with Dr. Axel Seltsam to expose the molecular diversity of the *JMH* gene. Presently there are six antigens identified from what was once believed to be a single, annoying antibody specificity. It remains to be seen what role

these antigens may play in the modulation of natural killer cell function.

Summary

John's dogged determination to work with and identify the HTLA group of antibodies has certainly led to many important discoveries. From a medical technology student to a world-renowned immunohematologist, John Moulds has come a long way (Fig. 2). And we, his students, friends, and collaborators, "stand up and cheer for this doer, this achiever, the one who recognized the challenge and did something about it" (paraphrased quote from Vince Lombardi). We are glad you did, and we will never forget your many scientific contributions.

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If a reagent can be made, John J. Moulds can make it

John J. Moulds has had a dramatic impact on blood bank technologists worldwide, making their lives easier when performing pretransfusion testing and problem solving. John's many contributions to the development of commercial reagents allow technologists to perform testing without the need for extensive preparation of reagents and without intensive method and technique applications. "Professionally developed and manufactured reagents for professional use that simplify the life of the blood banker ..."

On February 19, 2011, the John J. Moulds Reference and Scientific Support Laboratories were dedicated to John J. Moulds. This is a reiteration of the presentation, "If a reagent can be made, John J. Moulds can make it," delivered at this ceremony.

When I was asked in the fall of 2010 to participate as a speaker at the symposium and laboratory dedication of the John J. Moulds Reference and Scientific Support Laboratories at LifeShare Blood Centers, I was both humbled and proud of that opportunity to honor both a great friend and mentor for the many accomplishments he achieved throughout his illustrious career. However, before I go into the focus of my

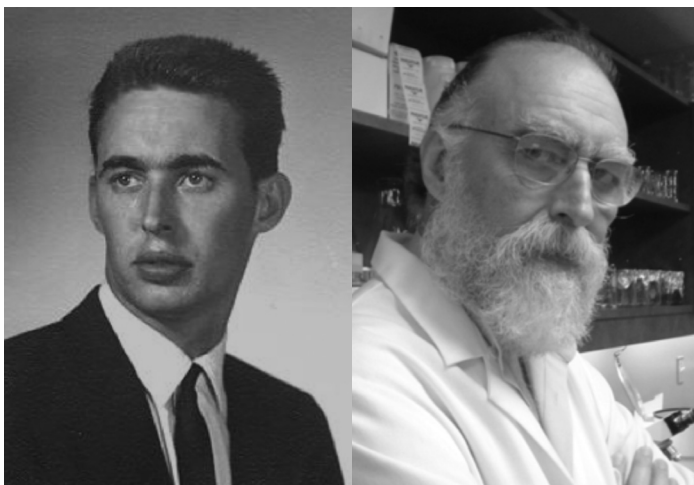


Figure 2. John as a medical technology graduate (left) and as Director of Scientific Support Services at LifeShare Blood Centers (right).

presentation at that dedication, I want to share a personal story, which I shared at the dedication as well, about John's influence on my career in the transfusion medicine industry.

My first encounter with John was via the phone. I was a full-time employee at the Medical College of Pennsylvania in Philadelphia, Pennsylvania, where I had just graduated from the medical technology internship program. My blood bank manager asked me to call Gamma Biologicals and ask for John Moulds to find out "what was wrong with these screening cells." One of the antibody screening cells was showing reactivity with serum samples from a few patients; subsequent antibody panel identification testing showed results with reactivity that appeared to have no antibody specificity. John kindly took the time to explain to me that the particular antibody screening cell expressed the Bg^a antigen and was likely involved because other cells that were tested on the antibody identification panel had "Bg" expression. As typical of the teacher in John, he patiently answered my questions and clarified what he could in light of the conversation being a phone discussion.

I walked away from that call impressed. About 2 years later, the sales representative from Gamma Biologicals happened by the hospital the week before the next meeting of the local antibody club, the Delaware Valley Blood Bank Club, which I had planned to attend and which featured John as the speaker. The representative made me an offer that I jumped at: after John was done speaking at the meeting, would I like to have a drink with him at a local "watering hole"? So after talking a little bit about the blood bank consultation service at Gamma and his travels talking about blood group serology and blood banking reagents, John asked if we could talk about something other than blood banking (I am sure that, as is the customary topic of conversation between sales representatives and customers, all John had done that day was talk about blood banking). So we talked about where we grew up, hobbies like hunting and fishing, and an old Corvette that he was trying to rebuild. Again, I walked away significantly impressed by the man and his career.

Because of these encounters with John, my experiences with teaching students, working on just about every antibody that was received in the hospital laboratory, and attending local and state blood bank meetings, I was hooked on blood banking. So my fate was sealed. His influence fueled my desire to work for a blood group reagent manufacturing company, focusing on blood group serology and the use of blood bank reagents. In 1983, I started my career in the commercial industry working for Biological Corporation of America (BCA) in the Blood Bank Consultation Service.

Over the years, John and I encountered each other at blood bank meetings. Always open to speaking at local, state, regional, national, and international meetings, John shared his knowledge with the blood bank masses.

In the early 2000s, I would get to work directly with John at Ortho Clinical Diagnostics. During more than 30 years of interactions with John, his willingness to share his knowledge, to discuss cases, and to be a mentor has had a great impact on my career. I am sure there are many out there who have a similar story to share about John.

Many of John's contributions to the development of commercial reagents have either simplified or solved challenges for the transfusion service or reference laboratory medical technologist in applying testing methods and techniques. With John's guidance and insights, and the assistance of colleagues at Gamma Biologicals, significant contributions were made to the introduction of these reagents.¹

Now on to the contributions that John has made to the blood banking world from a commercial blood bank reagent perspective that could be applied in testing by just about any medical technologist willing to follow instructions. So yes, "If a reagent could be made, John J. Moulds can make it." Figure 1 provides a timeline summary of the release of these various commercial blood bank reagents.

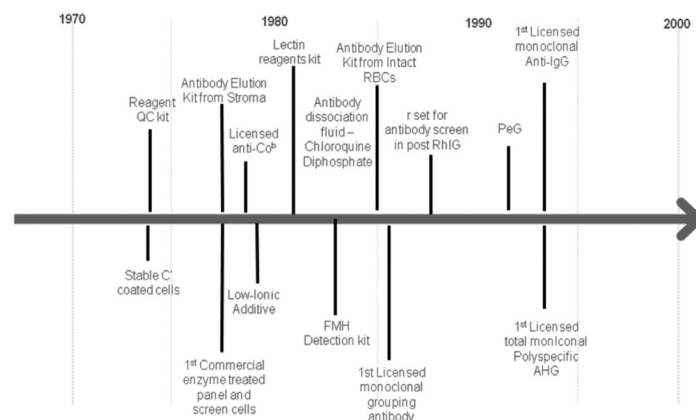


Figure 1. Adapted timeline for introduction of reagents.²

1970s

One of John's first uniquely developed commercial reagents in the early 1970s was stable complement-coated cells. Having to prepare special reagents, select the right donor red blood cell (RBC) to coat, and attempt to stabilize the complement coating on the RBC when preparing complement-coated RBCs are true challenges. The introduction of a commercially available ready-to-use stabilized complement-coated cell to quality

control anticomplement antiglobulin reagents certainly made it easy to assure that anti-human globulin reagents containing anticomplement activity were performing acceptably. No longer was it necessary to spend hours preparing materials, qualifying the “right” donor, coating the cells, and in the end having to quality control the prepared complement-coated cells.

Proteolytic enzymes and their effects on RBC structure and antigens have been extensively studied. Use of enzymes in blood group serology was limited to those experienced in their preparation and use. Variations in enzymes and treatment approaches created variability in observed test results. In the late 1970s, John, along with the consultation service and RBC production departments at Gamma, had prepared ficin-treated panels, and John shared them with other serologists in a few reference laboratories throughout the United States. After doing this several times during the year, John found the number of reference laboratories wanting to get in on the “enzyme panel” expanding. The first commercially available enzyme-treated panel was born. The Gamma ficin panel became popular because it eliminated the need for the special preparation required for enzymes, and for treatment standardization and it had stability, so that the product had a longer shelf life compared with the self-made preparations. Now not only reference laboratories but transfusion service laboratories could perform enzyme antibody identification panels using the commercial panel. Resolving some complex antibody problems with enzymes now became simpler and quicker, decreasing turnaround time.

The ability to remove the coating antibody from RBCs to identify it is a very useful tool for solving antibody problems associated with autoimmune hemolytic anemia, hemolytic disease of the newborn and fetus, drug-induced hemolysis, and hemolytic transfusion reactions. Elution procedures for preparing eluates were often limited to a safe but ineffective method such as a 56°C heat elution or choosing a chemically oriented method with potential dangers in its use. The discovery that an acidic solution could be used to elute antibody from RBC stroma created a safe, effective way to produce an eluate.³ John took this information and created the first commercially available elution kit, Gamma Elu-Kit I, which used a series of reagents including a solution of digitonin, a wash solution, an acid solution, and a buffer solution to create a popular approach to performing elution.

Later in the 1970s the introduction of the first licensed anti-Co^b, with John’s leadership, led to a stream of new reagent antisera to “rare antigens” and to a variety of blood group antigens during the next decade.

John followed with two reagents formulated to help enhance antibody detection and identification tests. The low-ionic-strength saline (LISS) solutions method, as described by Löw and Messeter,⁴ used RBCs that required the cells to be washed and suspended in the solution for use. John helped develop two reagents, Gamma LO-ION and Gamma N-Hance. The Gamma LO-ION product, an additive-based LISS reagent, used the traditional LISS solution along with a high-molecular-weight protein (polyvinylprolidine) to create a popularly used tube-based LISS additive. Gamma N-Hance used the traditional LISS-based approach combined with bovine serum albumin as part of the formulation. Both additive methods allowed for any properly prepared RBC suspension to be tested easily without special preparation of the cells in a LISS solution.

1980s

The 1980s brought a plethora of new innovative commercial reagents driven by John. Polyagglutinable cells are generally easy to define and identify with the use of the right lectins that react and agglutinate certain RBC antigens. John enjoyed working with cells that demonstrated polyagglutination. Making life easier for technologists working with polyagglutinable cells, John worked to produce and standardize lectin reagents for manufacturing. An abstract “Care and cultivation of *Vicia graminea*” by John and others in the early 1980s kept it all in the family, as even John’s mother, Edith, made a significant discovery.⁵ (Fig. 2.) *V. graminea*, the plant from which the seeds for the lectin are harvested, is actually in a dormant state when it looks as if the plant has died. Edith found that the plant starts to grow again in the spring to produce seeds (personal communication, Marilyn



Figure 2. Tony Casina and Edith Moulds at the dedication of the LifeShare laboratories to John Moulds in 2011.

Moulds). An anti-N lectin (*V. graminea*) was developed and commercialized through these efforts. In the early 1980s, the Gamma Lectin Kit, with its four lectins, *Arachis hypogaea*, *Salvia sclarea*, *Salvia horminum*, and *Glycine max* (soja), allowed the identification of the most common forms of polyagglutination. The kit delivered the convenience of a manufactured standardized set of lectins without all of the “grinding and refining.”

The presence of antibody on RBCs can be quite impairing when attempting to antigen type RBCs. The removal of antibody from direct antiglobulin (DAT)-positive RBCs had traditionally been attempted by gentle heat elution to effect removal of antibody from the RBCs. This method was time consuming and often did not work very effectively. The discovery that chloroquine diphosphate could elute antibody effectively from most RBCs without significantly affecting antigen structure led to John’s development of a manufactured reagent, Gamma-Quin. This reagent could be used to remove antibody from DAT-positive RBCs, which allowed the use of antiglobulin-based typing reagents to test these RBCs for other blood group antigens.

In the mid-1980s, John drove innovative improvements to the Elu Kit I elution kit by introducing the capability of eluting antibody from intact RBCs. This innovation eliminated the need to wash the stroma free of hemolysis created by the digitonin lysis of RBCs and further reduced the turnaround time to obtain a quality eluate. Additionally, the remaining intact RBCs could be used for antigen typing in the appropriate circumstances.

The middle 1980s brought two manufactured reagents, which addressed the needs of obstetrical patients: Gamma Fetal Bleed kit and Gamma r-set antibody screening cell. John and the consultation staff formulated the Fetal Bleed Kit based on the Sebring-Polesky procedure⁶ for fetomaternal hemorrhage testing. This kit made the evaluation of fetomaternal hemorrhage an easy-to-perform blood bank test. The introduction of the Gamma r-set screening cells, of which John directed the design, simplified dealing with patients, particularly obstetrical patients who had received Rh immunoglobulin and had anti-D, eliminating the need to perform standard antibody identification in these patients. This simplified the process of finding selected cells for testing when anti-D is present.

With the innovation in the 1980s of monoclonal antibody technology in blood group serology, John introduced the first monoclonal antiserum for blood group antigen testing, anti-He. He was instrumental in the introduction of additional

specificities to the Gamma-clone line of monoclonal antibodies, including anti-Mg, -M, -N, -Le^a, -Le^b, -P₁, and -K.

1990s

Unique new reagents continued appearing in the immunohematology testing market, flowing from a concept in John’s mind to a manufactured commercialized reagent. In the early 1990s John took a recently identified method for detecting and identifying blood group antibodies using polyethylene glycol (PEG), a test that Nance and Garratty⁷ published in the late 1980s, and produced a product that can be used in tube-based tests for antibody detection that showed greater sensitivity with some blood group antibodies. The combination of PEG and LISS solution led to the Gamma PeG reagent that delivered the sensitivity of PEG in a convenient commercially prepared reagent.

Later in the 1990s John revolutionized the way antiglobulin reagents are produced, introducing the first monoclonal-based anti-IgG that eliminated the need to use rabbit-sourced anti-IgG. This murine monoclonal antibody had unique specificity in that it did not detect antibodies of the IgG4 subclass, which have been shown to rarely have any clinical significance. Additionally, this monoclonal anti-IgG allowed for the development of the only total monoclonal polyspecific anti-human globulin (anti-IgG, -C3d).

2000s

John brought his innovative thinking and problem-solving skills to Ortho Clinical Diagnostics in 2000. Applying those skills, he helped resolve challenges with immunohematology products used worldwide and contributed to the development of new reagents.

It is obvious that John’s unique thinking and practicality afforded him the knowledge and wisdom to take complex technical processes and procedures and simplify them. That ability has led to the many unique immunohematology reagents that are used by medical technologists throughout the world to perform test procedures that normally would not be available to them.

I have only scratched the surface of the many reagents that John J. Moulds has had some hand in formulating into a medical technologist–friendly usable tool for immunohematology testing and problem solving. Professionally developed and manufactured reagents for professional use that simplify the life of a blood banker ...

John will be missed by the many people he touched with his knowledge, wisdom, and mentorship. Personally, John was a great friend and I will miss him tremendously.

Acknowledgments

I would like to thank Marilyn Moulds for her review and suggested improvements of this article.

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It was with great humility, admiration, and pleasure that I attended the symposium and dedication of the John J. Moulds Reference and Scientific Support Laboratories at LifeShare Blood Centers in Shreveport, Louisiana, on February 19, 2011. John had been a member of the editorial board of *Immunohematology* since 1997. During that time, John had contributed to the journal through his innovative input to the journal process and format at the annual journal breakfast meetings at the AABB conference as well as through his timely and thorough review of manuscripts submitted for possible publication. John's suggestion of providing serologic information in removable centerfold format in the journal will be implemented in this issue. His interest in the antibodies to

low-incidence antigens led to his being asked to write a review of these important but sometimes forgotten blood group antigens. However, despite his intentions, time did not allow for this publication. I was honored to present John with a plaque commemorating his many years of insight, inspiration, and service on the board of *Immunohematology* at the LifeShare laboratory dedication (Fig. 1). John will be long remembered for his many contributions to the journal *Immunohematology* as well as to the field of transfusion medicine.

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Figure 1. Cindy Flickinger presenting commemorative plaque to John Moulds at LifeShare Dedication in 2011.