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In this edition of *Immunohematology*, Dr. Phillip Sturgeon describes the beginning of automated blood typing. It is not often that one can read personal reminiscences of the origins of technical advances that revolutionized our field.

When I arrived at the Los Angeles Red Cross in 1978, a modification of Dr. Sturgeon’s machine was hard at work ABO and Rh typing more than 1000 donors per day. This machine was replaced around 1980 with a French machine (the Groupamatic) that utilized microplates. Eventually, this machine was replaced by a Japanese machine made by Olympus. More recent modifications of this machine are used for typing all donors at American Red Cross National Testing Laboratories.

From 1948 to 1960, Dr. Sturgeon was the Chief of Hematology Research at Childrens Hospital, Los Angeles. In 1960, he was made National Associate Director of the Red Cross Blood Program and Director of the western branch (in Los Angeles) of the National Research Laboratory. Soon after his arrival at the Red Cross, Dr. Sturgeon started a reference laboratory (Dorothy McQuiston was the chief technologist) and a rare donor file. By 1963 he had a file of 10,000 donors with unusual blood types, and he was satisfying requests from blood centers in other parts of the United States. His master plan was to have at least ten other Red Cross centers doing the same type of work. He left the Red Cross in 1966 to become Professor of Pediatrics and Head of the Division of Hematology at the University of California in Los Angeles (UCLA), a position he held until 1973. He then carried out research at Cedars Sinai Hospital until 1980. During that time, I was honored to be a coauthor of one of his papers about an IgA Rh autoantibody (*Transfusion* 1979;19:324).

In addition to his seminal work on automation, Dr. Sturgeon published many important immunohematology studies. He contributed to our understanding of the serology, hematology, and biochemistry of “permanent in vivo mixed field agglutination” (polyagglutination). He was the first to describe the Lewis blood group substance, Le\(^a\), and to describe the hematological abnormalities (e.g., stomatocytosis) associated with Rh\(_{null}\) phenotype.

Dr. Phillip Sturgeon is an extraordinary person and scientist. He is still contributing (by writing his scientific memoirs) while in his 80s, in retirement in Switzerland. In addition to his more than 122 scientific publications, Dr. Sturgeon has published articles on his experiences (e.g., skiing and trekking) in the Swiss Alps and the Himalayas. Selected titles of articles are: Mani Stones and Mantras; A Tibetan-English Beginner’s Dictionary (published in 1999); Shopping in Kathmandu (published in 2000); and a letter to the editor (published in 1999) with the fascinating title Abdominal Recollections (The Loo at Nepalgunj)! He also is working on a biography of his father, Rollin S. Sturgeon, Sr., who was one of Hollywood’s three movie pioneers.

In addition to all the above accomplishments, Dr. Sturgeon was awarded the Bronze Star and the Purple Heart in World War Two.

Young immunohematologists have to look no further than this man for an inspirational model.

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and
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Automation of blood typing was a thing of the future when I joined the American Red Cross (ARC) in Los Angeles, California, in 1960. Automation had found its way into a variety of medical laboratory procedures over the previous decade, but blood typing somehow had escaped. For each bottle of labeled blood released from a routine blood bank, the donors’ cells and sera had to be tested using sera and cells of known types. Dozens of methodical, repetitious, time-consuming—not to mention boring—hand manipulations made automation’s time long past due. Over the course of the next several years, not only was the need for automation met but a valuable tool was born, which opened up avenues of research applicable in both applied and basic medicine.

**Development of the Single-Channel Prototype**

Dr. Sam Gibson called me from his office at the ARC’s National Headquarters in Washington, DC, to inform me of a 3-year research project that Dr. Creighton McNeil, a physician consultant to the Red Cross’ Blood Bank in Salt Lake City, was engaged in with the Technicon Instrument Corporation (TIC), Chauncey, NY, the leading manufacturer of equipment for the automation of medical laboratory procedures. They had been attempting to adapt such equipment to the automation of blood typing, with their attention focused exclusively on the ABO system. The work had been financed by the ARC but the results of their efforts, though tantalizing, fell short of the mark. They had just notified Dr. Gibson that they had come to a final, joint decision to abandon the project, but to publish the results of their efforts in the hope that someone else would pick up the research.

Mr. William (Bill) Smythe (Head of Research and Development, TIC) showed me to his laboratory and explained the prototype of the blood-typing AutoAnalyzer. “Living spaghetti bolognese” was the first thought to cross my mind. There it was, a jumble of fine plastic and glass tubes that were squirming about as rock roller pumps circulated all of the ingredients necessary to a blood-typing reaction through the maze. Due to my experiences as a boyhood backyard mechanic who had worked on Model T Fords and other antique cars, and to Mr. Smythe’s lucid explanations of the principles involved in continuous-flow automation used by the AutoAnalyzer, I was able, within a brief time, to grasp how the ungainly machine typed blood. The impasse that Dr. McNeil and TIC had not been able to escape was that the $A_2B$ blood type slipped through as type B.

The prototype machine had the sensitivity necessary to detect types A, B, and O, as well as the weaker $A_2$ variant; however, when $A_2$ is a component of the AB complex, $A_2$ becomes even weaker, and the prototype machine lacked the sensitivity to detect it. An $A_2B$ donor’s blood would come out as type B, and were it to be given to a type B recipient, it would result in a transfusion reaction—possibly even a fatal one. Despite their 3 years of trying, type $A_2B$ continued to elude Mr. Smythe and Dr. McNeil.

Mr. Smythe gave me a demonstration of how, using the anti-B typing serum, the machine gave correct reactions, i.e., negative with type O and A cells, positive with type B and AB cells. With anti-A serum, there were...
the expected negative reactions with type O and B cells and all type A cells gave positive reactions. However, some type AB cells did not; those were A2B cells. The plan, following that demonstration, was to pack up the machine and ship it out to Los Angeles, where research on increasing its sensitivity would be undertaken.

At that point, I asked Bill Smythe to let me have a closer look at all of the reactions that were taking place as the test ingredients flowed into, and through, the machine. I followed them closely as they were progressively mixed in small glass coils and pumped along plastic tubes, and as agglutinates that formed were allowed to slowly settle out in large glass reaction coils. It was the latter reaction—that of agglutination—that was not taking place with the A2B cells. With the traditional manual method of testing, in which a drop of a dilute suspension of red cells was mixed on a glass slide with typing serum, the reaction took place in a few seconds. “What was the difference? Where along the way through the machine was the sensitivity being lost?”

The typing serum being used in the machine was the same as that used in the manual test. The volume of serum to the volume of cell suspension being tested was also the same, and the degree of mixing and the incubation time were all in favor of increased sensitivity in the machine. However, a closer look revealed one significant difference. In the traditional manual test, a very dilute cell suspension in saline solution is prepared from the whole-blood sample before mixing the cells with the typing serum. With the machine, the cells were being taken directly from the whole-blood sample, a very heavy suspension of cells, and were being mixed directly with the typing serum. Thus, the volumes were essentially the same as those used in the manual test, but the proportion of cells in relation to the antiserum was in great excess.

I drew Mr. Smythe’s attention to this discrepancy, and he agreed that it would be a simple matter first to draw the whole-blood aliquot into a dilution circuit and then to resample from that circuit into the prototype machine. He felt he could get it set up in a few minutes. Based on my experience with the manual procedure, I suggested a dilution of the whole-blood sample (which characteristically is approximately a 50% cell suspension) to a 2 percent suspension, a 25-fold dilution. Thus the strength of the typing serum, in proportion to the number of cells, would become 25 times as potent. Before the morning was out we had the results. An example of an A2B blood gave unequivocally clear-cut reactions. The breakthrough had come. The Auto-Analyzer was indeed practical for typing in the ABO blood group system, and, if it could be further refined to work for the more exacting Rh system, automation could be introduced into blood banking.

A prototype machine, with the above modification, plus some additional mechanical refinements, was sent to the Los Angeles ARC. There we made fine adjustments to the environment within the machine in which the agglutination reaction took place. Those included adjustments in the concentration of the salt solution, cells, and antiserum; length of incubation time; temperature; etc.; or, in laboratory parlance, in the necessary serologic conditions, all of which added further to the sensitivity of the machine. Twelve type A2B bloods all gave strongly positive results; in fact, among them was a very rare and extremely weak AB variant, type A3B, and it gave a positive reaction as well. However, for the more stringent demands of Rh blood typing, those improvements were not enough.
In ABO automated typing, as well as in the traditional manual testing method, the most favorable serologic conditions for the agglutination reaction to take place are obtained with the red cells in a saline suspension. The typing serum is also diluted in saline; that method is known as the saline agglutination test. With the discovery of the Rh factor, it became evident that, with the saline agglutination test, Rh positive cells would not give a positive reaction with most Rh antisera—even those that came from patients who had had fatal Rh blood transfusion reactions. Similar contradictions were soon to emerge with most of the other blood group systems coming to light at that time.

Attempts to find the laboratory (serologic) conditions that would consistently detect these relatively obscure blood types followed four main courses. One was to carry out the test with a very heavy suspension of cells in their serum and with the antiserum diluted in a strong concentration of albumin, the albumin agglutination test. It improved the sensitivity but still fell short of that required to detect D⁰. Another method was to allow the interaction of cells and antiserum to take place in saline solution and then, following several careful washings of the cells, to test them for adsorbed antibody with a rabbit antiserum that reacted in careful washings of the cells, to test them for adsorbed antibody with a rabbit antiserum that reacted in saline—the antiglobulin, or Coombs, test. This proved to be the most sensitive of all; however, it held little or no promise of being adaptable to automation, at least in a continuous-flow system. A third method was to reduce, in the medium in which the reaction is taking place, the electrostatic repulsive forces (forces that tend to keep the cells and antiserum apart). Changes in the salt concentration produce such an effect, as does the addition to the medium of large-molecular-weight substances such as gum acacia or polyvinylpyrrolidone (PVP); that is, macromolecular substances. The fourth method was to reduce the electrostatic repulsive charge on the cell surface by a preliminary treatment of the cell surface with protein-digesting enzymes, the proteolytic enzyme test. It was self-evident that the latter two would be readily adaptable to the continuous-flow system of the AutoAnalyzer, hence quite appropriate for testing for weak Rh variants and for other blood group systems.

Thus the first-stage ABO-cell-dilution circuit was modified to include an enzymatic treatment of the cells with ficin, an enzyme from figs. The ficinized cells were drawn off into the second stage and split into the ABO circuit and the Rh circuit, where Rh typing serum was introduced. It worked; Rh positive cells reacted well, including several types of the weaker variants. But the weakest of them all, D⁰, did not. This was overcome by taking advantage of the third option listed above. Gum acacia was the macromolecular substance used, and D⁰ cells reacted well. Thus, the A,B hurdle had been cleared and now the Rh and D⁰ hurdles were also cleared; automated blood typing, as required for routine blood banking, was at last a reality! Moreover, beyond the needs for routine blood banking, it was implicit in the above developments that the way was now open for the application of AutoAnalyzer continuous-flow technology to typing of antigens in other blood group systems, as well as to fields of laboratory medicine and medical research.

**Transition from the Single-Channel to the Multichannel Prototype**

Whatever may have been the minute and precise details of how automation of blood typing and its reporting evolved, its research had, by the end of 1963, reached the point where the single-channel prototype—one capable of performing but one test—had to be expanded into “...a multi-channel machine which can integrate all of the results [from] the several tests being performed upon a single sample of blood.”

The task sounded like a straightforward problem of mechanics; it simply required eight duplicates of the original prototype to be coordinated into a simultaneous operation. But when that first multichannel prototype was put to work, a host of problems emerged. To keep the eight channels in phase with each other (in order that all of the tests from a single sample of blood came out on the recorder simultaneously) required modification in the flow circuitry and some further serologic refinements. To keep glass tubes and coils clean and to prevent plug formation along the way, various wash solutions (detergents) were tried.

The serologic modifications essentially fell into the domain of the ARC in Los Angeles, and the mechanical modification fell to Mr. Smythe at TIC. But, by strange coincidence, the solution to a mechanical problem, which was taking on insurmountable proportions, fell into my hands. The probe that aspirated the cells from the original blood sample could become plugged by tiny blood clots that would occasionally form in the original sample. Then, in normal operation, during the interval between samples, when the probe was in the wash position and aspirating saline solution, the plug
would not release. Thus, from all subsequent blood samples, no further results could be obtained; in effect the machine was irreversibly shut down. Attempts at preventing or dissolving the plug clots with modifications in the washing fluids failed.

It occurred to me that the practical skills of a plumber had to be brought to bear on the problem. Why not try to keep the line clear by reversing the flow during the wash phase rather than introducing various cleaning solutions; i.e., to blow the plug back out of the probe. Knowing there were electronic valves that could automatically change flow directions, I brought this solution to the attention of Mr. Smythe. I suggested that he incorporate in the sampling line, immediately beyond the sample probe, a T-connection with a solenoid valve below. Then, between samples, in the washing phase, the valve could be programmed to direct a large quantity of saline solution into the system. That would come from a pump that would supply the amount needed farther along in the circuit and, in addition, a large excess, which in turn could only escape back through the probe. That was easier said than done, but Mr. Smythe took care of the details, and it worked.4

This was not just a glitch in AutoAnalyzer technology; the backwash principle now made it possible to present the device with whole-blood samples, as traditionally collected during routine blood donations. There would not have to be any extraordinary manipulations or precautions at the time of collection and, once the samples were in the laboratory, no preliminary manual loading into special sample cups would be required, nor would there have to be any washings or filtering. It held promise of permitting efficient testing of large-scale whole-blood sample collections from a normal blood-donor population. This would be the case not only for routine blood typing but also for research that required a large number of samples from normal populations. This little episode lingers in my memory as a quaint way in which knowledge that was not taught in medical school but was critical to medical research had to be called upon. Furthermore, it leaves me with some misgivings that, whatever may have been my contributions to the development of automated blood typing, the insertion of that T-fitting, in fact, may have been my most significant!

As the host of problems generated by the multichannel machine were overcome, a series of papers from my laboratory, identifying them and giving solutions, was published.57 One of the remaining problem areas had its origin in the machine’s two pieces of electronic equipment—the colorimeter and the recorder; there was one of each for each of the eight channels—16 in all. The colorimeter made an electronic measurement of the hemoglobin released from the red cells remaining after the agglutinates had been drawn off. The recorder traced out, as a series of separate peaks, the amount of the remaining hemoglobin detected by the colorimeter. A peak of full height meant a negative reaction, i.e., no agglutination had taken place. A flat valley meant a strongly positive reaction, i.e., all of the cells had been agglutinated and discarded. Peaks of intermediate height were a measure of agglutination reactions of intermediate strengths. With these two devices serving as the sensors of the blood typing reaction, the machine became known as the colorimeter-recorder unit. The interpretation of the tracings had to be done by visual inspection of the peaks and valleys; further processing of the data remained a clerical task as well.

At this juncture it became evident that at the rate at which the AutoAnalyzer could type blood, there would be a valuable saving in labor costs. A machine was put in service at the New York-New Jersey Red Cross Blood Center, where 10,000 bloods were tested in parallel with routine blood banking operations. It was observed that faults related to mechanical operations were the most frequently encountered, with incorrect phasing being the most common and time-consuming problem. The AutoAnalyzer colorimeter-recorder unit was determined to be a useful addition to the blood donor center’s routine.8 Nevertheless, it was also becoming worrisome that the complexity of the machine, with its demands for constant monitoring and adjustment of the phasing of the channels, was threatening to make the multichannel colorimeter-recorder unit too complex—and therefore impractical for routine blood bank use.

With those concerns in mind, it was Mr. Smythe alone who overcame the obstacle. In another application of Technicon Instruments, there was a very simple device that used a continuously moving broad belt of filter paper, and he recognized that it could be used to great advantage as the sensing system for blood typing. The agglutinates would be deposited on the filter paper for manual reading, and all of the associated complexities of manifolding required to get the remaining cells to the relatively ponderous colorimeters and recorders, including the latter two, could be done away with. Thus, a drastically revised system was devised. The serologic conditions did not have to be changed. The
agglutinates from each circuit were deposited as bright-red granular smudges in eight parallel rows on the moving filter paper. The nonagglutinated cells were drawn through the paper by vacuum and discarded. The absence of a smudge meant a negative reaction, i.e., no agglutination had taken place. A well-demarcated smudge meant a positive reaction, i.e., all of the cells had been agglutinated and were trapped on the filter paper. Further quantification of the smudges was not reliable; they essentially had to be read as either positive or negative.

A comparative study of the proficiency of the two machines was made by testing 3040 samples in the colorimeter-recorder machine and 4749 samples in the filter-paper machine. The latter proved to be faster, more efficient, and more reliable, and the consumption of reagents was one-third as great. Further, it was far less intricate, it had much less exposure to electrical and mechanical failures, it had less difficulty in keeping in phase, and its records were easily read and interpreted visually. It was estimated that two technicians operating one machine would be able, in one 8-hour day, to complete the ABO and Rh typing of all of the bloods collected by even the largest center in the world. Production models were soon under field trial in three American Red Cross Centers.8,9

Subsequent History of the AutoAnalyzer and New Technologies

It goes far beyond the scope of the present work to attempt a comprehensive history of automated blood typing subsequent to the events set out above. However, from the perspective of today's course of events, that is, one that sees the latest technological marvels almost outdated the day they reach the market, the history of automation did not follow an unusual pattern, albeit perhaps at a more relaxed pace. Sixteen years after those original publications on automation, it was estimated that 720 AutoAnalyzers had been sold.10 Also of considerable note was that it was only 6 years later that the Groupamatic appeared. That was an entirely new device designed in France at the Centre National de Transfusion Sanguine, in Paris. This was more of a robotized version of a manual test procedures; however, it worked well and had great speed, and data processing was readily incorporated in the system. To compete with the latter data processing capabilities, TIC introduced a new version of its machine, the Auto-Grouper, which similarly included a laser for reading sample numbers and integration of those with machine-interpreted results.11 "These two rival technologies for blood group automation and antibody quantitation were in fairly widespread use throughout the world in the mid-1970s."11 Suffice it to say, the problem of data processing proved to be a vastly more difficult one to solve than had been contemplated originally and, in retrospect, I am grateful indeed that it was my fate not to be drawn into it—hardly a field for one with a degree in medicine!

The late 1980s saw the introduction of yet another advance in technology, the Olympus, which used a unique micro-plate technology that does not require centrifugation.12 Dr. George Garratty, Scientific Director of ARC Blood Services, Southern California Region, recently informed me that these machines are in current use in the ARC National Testing Laboratory and that the AutoAnalyzer was replaced about 1980.13

Thus, in the long view, it was in the range of 60 years after the discovery of blood types that automation was introduced into blood testing, and it took another 25 years for it to be perfected. Although contributions to the latter came from the efforts of scores of investigators working in laboratories of many nations, it was in the ARC and in the TIC laboratories that the pioneering discoveries were made. After a faltering start, it was from my colleagues' and my research that the technology entered a phase of rapid growth; this was essentially the opinion given by Peoples: "The real breakthrough in practical automation for the blood donor center occurred when Sturgeon and co-workers used polyvinylpyrrolidone...as a potentiator."11

However, I believe that the time of the breakthrough antedated the introduction of PVP—so that it could be pinpointed more precisely; i.e., the time when, in 1960, Mr. Smythe and I were studying the faltering prototype machine in his laboratory. It was then that I suggested that the proportion of cells to antiserum needed major correction; and Mr. Smythe immediately took care of the problem. Because of that correction, we were privileged to observe for the first time a type A,B blood give a positive reaction! Whatever may have been the short- and long-term consequences of that crucial event is probably difficult to say. However, it would not be too far-fetched to propose that, had the consequences not occurred, TIC would not have become involved in the automation of blood typing and the introduction of automation to blood banking could have been delayed for several years.
Serologic aspects of treating immune thrombocytopenic purpura using intravenous Rh immune globulin

C.M. Savasman and S.G. Sandler

In patients with immune thrombocytopenic purpura (ITP), IgG autoantibody-coated platelets are phagocytized by mononuclear macrophages, primarily in the spleen. Intravenous Rh immune globulin (IV RhIG) has been used since 1983 to treat D+, non-splenectomized patients with ITP. The beneficial therapeutic effect of IV RhIG is attributed to competitive inhibition of phagocytosis of IgG-coated platelets by IgG anti-D-coated D+ red blood cells (reticuloendothelial or Fc receptor blockade). Following infusions of IV RhIG in D+ ITP patients, the direct and indirect antiglobulin tests become transiently positive, reflecting passively transferred anti-D and other alloantibodies that were present in the infused IV RhIG. These consistent and predictable serologic findings contrast with the inconsistent and weak anti-D reactivity observed when D- women are treated with relatively small doses of intramuscular RhIG for Rh immunoprophylaxis. The pathophysiology of ITP and the effect of infusing IV RhIG in patients with ITP are illustrated in this review, using computer-generated figures. Immunohematology 2001;17:106–110.
Easy method for determining the frequency of $O^1$ and $O^2$ alleles in Brazilian blood donors by PCR-RFLP analysis


Serologic ABO blood typing is routinely performed using anti-A and anti-B sera to distinguish four phenotypes (A, B, AB, and O). Restriction fragment length polymorphisms (RFLPs) and DNA sequence studies offer the possibility of direct ABO genotyping. We used polymerase chain reaction-RFLP analysis to determine the frequency of $O^1$ and $O^2$ alleles in 82 unrelated blood donors in São Paulo, Brazil, known to be group O. Genomic DNA was extracted from blood leukocytes by a modified salting-out method. Different genotypes ($O^1O^1$, $O^1O^2$, $O^2O^2$) were identified after digestion with restriction enzymes KpnI, HpaII, and AluI, followed by agarose gel electrophoresis. Of 82 samples analyzed, 74 were $O^1O^1$, 7 were $O^1O^2$, and 1 was $O^2O^2$. These results showed the frequency of $O^1O^1$, $O^1O^2$, and $O^2O^2$ genotypes to be 90.24 percent, 8.53 percent, and 1.22 percent, respectively, in blood donors in São Paulo, Brazil. *Immunohematology* 2001;17:111–116.

Case report: four donors with granulocyte-specific or HLA class I antibodies implicated in a case of transfusion-related acute lung injury (TRALI)

A. Davoren, O.P. Smith, C.A. Barnes, E. Lawlor, R.G. Evans, and G.F. Lucas

A 54-year-old female patient with a history of chronic liver disease and portal hypertension was admitted for an elective cholecystectomy. Preoperative evaluation revealed a prolonged prothrombin time of 17.4 seconds (control 12 to 15.5 seconds). Six units of fresh frozen plasma (FFP) were prescribed after failure of correction of the coagulopathy with intravenous vitamin K (10 mgs). During infusion of the fifth unit of FFP, the patient became acutely dyspneic. Arterial blood gas analysis revealed marked hypoxemia (PO$_2$ 6.58 kPa) and the chest X-ray showed new diffuse bilateral alveolar infiltrates. The patient remained hypoxemic with unstable oxygen saturations over the following 7 days, during which time she required 60 to 100 percent oxygen administered by face mask. Intravenous methylprednisolone (200 mgs) was given for 5 days. Mechanical ventilation was not required. The lung infiltrates gradually cleared over 3 to 4 days and the patient showed clinical improvement after 1 week. Four of the donors of the implicated units of plasma were female and all had a history of pregnancy. Two donors had HLA class I antibodies and two had granulocyte-specific antibodies detectable in their serum. In crossmatch studies, granulocyte-reactive antibodies from two donors bound to granulocytes from the patient, which suggested that these antibodies were clinically relevant. These clinical and serologic findings support a diagnosis of transfusion-related acute lung injury (TRALI). *Immunohematology* 2001;17:117–121.
One thousand seventy antibodies detected only by a 2-stage papain test: wanted and unwanted positive reactions

D. CASTELLÀ, J. CID, M. PANADÉS, AND C. MARTÍN-VEGA

Despite the wide use of the antibody detection test for unexpected antibodies, controversy still remains regarding the use of enzyme-treated red blood cells. Over a 6-year period, 72,573 samples from 49,863 patients submitted for pretransfusion compatibility testing were examined for unexpected antibodies. The antibody detection tests included a low-ionic-strength solution (LISS) indirect antiglobulin test and a two-stage papain (2SP) test. One thousand and seventy of the 2267 (47%) antibodies tested by 2SP were reactive only by the 2SP test. Overall, the 2SP test detected only 0.6% of antibodies considered to be clinically significant (10 examples of anti-c and 2 examples of anti-e). The slight additional safety provided by detection of clinically-significant antibodies is overshadowed by the high number of clinically-insignificant antibodies detected by the 2SP test. Immunohematology 2001;17:122–124.

Switching donor cells as a major source of error in compatibility testing

B.J. PADGET AND J.L. HANNON

The most likely cause of fatality in blood transfusion is transfusion of the wrong unit of blood to a patient. This type of error is usually attributed to improper patient identification at the time of sample collection or transfusion. A retrospective analysis of the results of an external proficiency testing program identified a common source of error occurring during laboratory testing that has not been previously reported. Results were analyzed when major errors were assigned to laboratories for obviously switching donor units in compatibility testing and/or subsequent investigation. In 24 surveys sent to extended testing (Level A) laboratories and 18 sent to basic testing (Level B) laboratories, the antigenic composition of the two donor cells made it possible to determine whether the cells had been switched. Seven errors were assigned to Level A participants for switching donor units during testing, constituting 38.9 percent of the 18 major errors assessed. Level B participants were assigned eight errors for switching donor units, 26.7 percent of the 30 major errors assessed. Approximately one-third (31.3 percent) of major errors committed on 42 proficiency testing surveys were caused by switching of donor cells during compatibility testing. This type of error may result in transfusion of an incompatible donor unit. Immunohematology 2001;17: 125–129.