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New protocols in serologic testing: a review of techniques to meet today's challenges

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Introduction

In 1945, Coombs et al.¹ described the indirect antiglobulin test (IAT), and Diamond and Abelson^{2,3} described albumin, as a potentiator for the detection of "incomplete" or IgG antibodies. It has been 55 years since Coombs's and Diamond's revelations. The classic saline IAT is still viewed as the gold standard. According to the literature, an IAT incubated for 60 minutes at 37°C in saline will provide 99 percent of antibody uptake onto red blood cells (RBCs) from any sample containing an appropriate IgG antibody.⁴ If the incubation time is reduced to 45 minutes, still in saline, there will be 95 percent uptake of antibody.⁵ All subsequent methods and enhancement media were developed from this reference point. For example, low-ionic-strength solutions (LISS) were developed and used to speed up the rate of antigen-antibody association so that the optimal range of antibody uptake (95 to 99%) could be achieved using 15- to 30-minute incubations. Methods such as a capillary technique and potentiators such as Polybrene[®] have come into and out of favor. Over the years it has become increasingly clear that no single method is perfect. The IAT, in its traditional test-tube format, has, however, proven invaluable in the detection of clinically significant RBC antibodies and is still used by many as the test of choice.

As good as the IAT is, there are many factors that can affect the reproducibility and reliability of this gold standard. Inherent to this standard test-tube system are the undesirable effects that arise from the necessity of the wash step prior to the addition of anti-human globulin. Low-affinity immunoglobulins bound to RBCs may elute off during the wash procedure. Techniques for reading and grading reactivity are difficult to learn, and it may be difficult for staff to maintain competence in low-volume laboratories. The unstable endpoints for the tube method can make reading, grading, and

interpreting tests almost impossible. Alternatives have been developed to address some of these issues. In particular, some of these relatively new methods represent attempts to control the undesirable effects of the washing step, individual reading techniques, the level of expertise needed to grade results accurately, and the unstable endpoint, as well as to optimize pH, ionic strength, the serum-to-cell ratio, and the use of automation techniques. By controlling these variables, ease of testing, better reproducibility, and better reliability of test results can occur.⁶

To maintain the quality of testing, documentation, and overall patient safety required by regulatory agencies, it has been crucial to develop new methods. The alternative methods currently licensed for use in the United States are commonly called gel tests, i.e., RBC-affinity column technology, and solid-phase technology. The purpose of this review is to summarize the principles of the different methods, their advantages and disadvantages, and the automated equipment available.

Principles of the Tests

The gel test

The gel test (ID-MTS; Ortho-Clinical Diagnostics, Raritan, NJ) was released in Europe in 1988 and was available in the United States in 1995. The technology was developed by Lapierre et al.⁷ and is manufactured in the United States by Micro Typing Systems, Inc. The basic principle of the gel test is that, instead of a test tube, the serum and cell reaction takes place in a microtube consisting of a reaction chamber that narrows to become a column about 15 mm long and 4 mm wide. Each column contains about 35 μ L of a dextran acrylamide gel prepared in a buffer solution such as LISS or saline. The gels may also contain other

elements: preservatives such as sodium azide, sedimenting agents such as bovine serum albumin, and, in some cases, specific reagents such as anti-IgG or other RBC-specific antisera (ABO and D). When gels are to contain specific reagents, the reagents are added to the gel during preparation by the manufacturer before the microtube is filled. Thus, the reagent is dispersed throughout the length of the gel column. The gel column is about 75 percent packed gel and 25 percent liquid. Six of these microtubes are embedded in a plastic card to allow ease of handling, testing, reading, and disposal.⁸

The manufacturer's insert outlines the following procedure to perform an IAT. Using a gel microtube that contains IgG, 25 μL of serum or plasma and 50 μL of LISS-suspended RBCs at an 0.8 percent concentration (pipetted separately) is added to the reaction chamber of the microtube. The microtube is incubated at 37°C for 15 minutes, then spun for 10 minutes in a dedicated centrifuge at approximately 70 $\times g$. During centrifugation, RBCs in the reaction chamber are pulled into the gel column. The serum mixture along with unbound serum proteins lack the weight necessary to be pulled from the reaction chamber and therefore do not enter the column where they could potentially neutralize the anti-IgG in the gel. Sensitized RBCs will pass through the upper part of the gel column and agglutinate in the presence of anti-IgG. Agglutinated RBCs are too large to pass through the gel matrix, so they are trapped at various places within the gel, depending on size of the agglutinates, while unagglutinated RBCs slip easily through the gel and pellet at the bottom of the microtube.

After centrifugation, positive reactions are indicated by RBC agglutinates trapped anywhere in the column of the gel. Positive reactions can be graded from 0 to 4+. A 4+ reaction is indicated by a solid band of RBCs on top of the gel. A 3+ reaction displays agglutinated RBCs in the upper half of the gel column. A 2+ reaction is characterized by RBC agglutinates dispersed throughout the length of the column. A 1+ reaction is indicated by RBC agglutinates mainly in the lower half of the gel column with some unagglutinated RBCs pelleted at the bottom. Negative reactions display a pellet of RBCs on the bottom of the microtube and no agglutinates within the matrix of the gel column. Mixed-field reactions can also be observed in the gel test. These reactions are more commonly encountered during RBC typing procedures rather than during serum or plasma testing methods, but in either case they are easy to recognize. Antigen-

positive RBCs, in this case, are completely agglutinated by the specific antisera present and they lie at the top of the gel, whereas the remainder of the RBCs that are antigen negative do not agglutinate and pellet at the bottom.

RBC-affinity column technology

The other column technology test (ReACT[®]; Gamma Biologicals, Houston, TX) was released for the United States market in 1997.^{9,10} Recently, Gamma Biologicals has become a subsidiary of Immucor, Inc. (Atlanta, GA). The ReACT test detects IgG-sensitized RBCs in an immunologically active matrix. In the ReACT test system, six or eight microcolumns approximately 12 mm in length and 3 mm in diameter are molded together in a plastic strip. Each microcolumn consists of a wider test chamber that narrows to a microtube. The microtubes are filled with an immunologically reactive agarose gel, which is physically separated from the contents of the test chamber by a viscous serum-cell barrier. The immunoreactive matrix is composed of sepharose gel particles to which protein G is covalently bound and sephacryl particles to which protein A is covalently bound. Protein G and protein A are bacterial proteins from *Streptococcus* group G or C and *Staphylococcus* group A, respectively, that bind specifically to the Fc portion of IgG molecules. Protein G has a high affinity for all subclasses of IgG molecules, whereas protein A will not detect IgG3. The blend of sepharose-protein G and sephacryl-protein A results in a layer of protein G at the top of the gel column. This layering effect helps clearly distinguish and visualize positive reactions.

The manufacturer's insert outlines the following procedure to perform an IAT. One drop or 50 μL of 0.8 percent LISS-suspended RBCs and one drop or 50 μL of serum or plasma are added to the test chamber. The entire test strip then is incubated at 37°C for 15 minutes and then centrifuged using a programmed cycle for approximately 3 minutes. During centrifugation, the RBCs in the reaction chamber are pulled through the viscous barrier into the agarose gel to which protein G and A are bound. The serum mixture remains in the test chamber because it cannot pass through the viscous barrier. This avoids the neutralization of protein A and G by unbound serum immunoglobulins. Any RBCs sensitized with IgG will adhere near the top of the column to the immunoreactive agarose gel. If there is no IgG bound to the RBCs, they migrate through the gel and are deposited at the bottom of the microtube.

Positive reactions are therefore visualized by a RBC band of any size left near the top of the agarose gel column. Positive reactions may be graded as strong positive, positive, weak positive, and negative or by the 4+ to 0 grading system, depending on the size of the RBC band at the top of the gel. Negative reactions result in the absence of a band of RBCs at the top of the gel column and deposited RBCs at the bottom of the microtube. Negative tests can be quality controlled by the addition of IgG-coated “check cells,” if desired.

Solid phase technology

The RBC solid-phase adherence assay is commonly referred to as “solid-phase” (Capture-R[®]; Immucor, Inc.). The test has been in use since the early 1980s in Canada^{11,12} and in the United States since shortly thereafter.^{13,14} The solid-phase platform is a 96-well microtiter plate that can be used as a whole or broken down to individual 8-well strips. The plates are stored in sealed foil pouches with a moisture indicator. If the indicator has turned pink, the plate has been exposed to too much humidity and should be discarded. The commercial product can be purchased in several formats. The wells can be coated with the RBC membrane lysate from either pooled, two-cell, or four-cell antibody screening cells selected for antigen combinations in the same manner as the liquid product or with membranes that compose a 13-cell panel. One can also purchase plates that are coated only with the proprietary adhesive and prepare a custom antibody screen or select-cell panel using liquid or frozen intact RBCs. The reagent that replaces anti-IgG in this method is the Indicator RBCs (Immucor) that are labeled with IgG directed against human IgG. All the Capture-R[®] kit products include a LISS additive with a protein indicator that changes the reagent’s color from purple to blue when serum or plasma is added to a well. Positive and negative controls that are used to validate each test run are also included in the kit.

To follow the manufacturer’s directions to perform an IAT, add two drops of the LISS additive to each well being tested. One positive and one negative control well must be included with each batch tested. The sample of choice is EDTA plasma, but serum can be used. One drop (50 μ L) of patient sample is added to each test well. One drop of control is added to the appropriate well and the plate frame (containing 1 to 12 strips) is put on a plate mixer or is lightly tapped at the corners until the blue color of the LISS and sample mixture is visible in all the wells. The plate frame is

incubated at 37°C for 15 minutes and then the mixture is washed out of the wells. This can be done with a squeeze bottle (3 washes and blot), a hand-held strip washer, or an automated plate washer with predetermined programs. In all cases, the plate is blotted dry onto absorbent material and two drops of indicator cells are added to each well. The plate or strips are centrifuged for 3 minutes at 350 \times g in a centrifuge and read. A positive reaction is indicated by a smooth coating of indicator cells over the round surface of the bottom of a well, which shows that the indicator cells have bound to antibody. A negative reaction is a button of cells at the bottom of the well. Grading can be performed using the classic 0 to 4+ scale. ABO and Rh testing can be performed in strips as well, but these tests still use a direct agglutination method, and a special coating on the bottom of the well is not required.

Advantages and Disadvantages

Although each of the three methods look very different from each other and from tube testing, they have all been designed to meet the same testing needs. They all have moved from glass to plastic as a safer choice of material and they all have standardized endpoints in preparation for automation. Within each method there are advantages and disadvantages due to their unique design.

The gel test

Advantages of the gel test include small sample size, decreased variation in volume delivery, greater uniformity between repeat tests, no cell washing step, and decreased technique dependence. The gel test is also easy to learn, since there are relatively few steps in the procedure and it provides a clear, easy-to-read, stable endpoint. When the microtubes are covered and refrigerated, the gel cards can be read with accuracy for at least 24 hours after testing. Sensitivity and specificity of gel testing have been found to be comparable to the tube LISS-IAT.

There are a few disadvantages. For reference laboratories, batch testing is less of an option because of urgency, timing, and variation in samples. Therefore, the efficiency made possible by batch testing is often lost. Complexity of antibody identification usually requires multiple runs of selected cells. Although an 0.8 percent panel of RBCs is commercially available, any other test cell that needs to be tested must be prepared as an 0.8 percent concentration in the appropriate diluent prior

to use. This is more cumbersome than adding the cells right from the vial into a test tube. The ABO and Rh typing may also have a disadvantage, depending on the laboratory's needs. The RBCs must be incubated in Diluent 1 for 10 minutes prior to centrifugation in the appropriate card. Therefore, it takes a minimum of 20 minutes (10 minutes incubation and 10 minutes centrifugation) to perform an ABO and Rh test. The test-tube method still wins on this one with ease and speed. Although direct antiglobulin tests (DATs) can be done on the gel system, only a DAT using anti-IgG is available. At this time, gel cards containing polyspecific anti-human globulin or anti-C3d are not available. Finally, rouleaux and incompletely clotted samples may cause patterns that resemble positive reactions.

RBC-affinity column technology

There are advantages to the ReACT system. Compared to the traditional tube testing, the technology is very simple, with few procedural steps. It requires a smaller sample size, does not require a cell-washing step, and goes directly from the incubation step to a 3-minute centrifugation and the final reading. Following interpretation of the centrifuged test, negative tests can be checked for reactivity by the addition of known IgG-sensitized RBCs. The reactions are relatively easy to read and are stable for up to 4 hours when the strip is stored upright. If the test strip is sealed to prevent evaporation and stored upright, the reactivity can be read up to 3 to 5 days after testing is completed. Clinically benign antibodies of the IgM class that can often interfere in other test systems, leading to lengthy and costly investigations, are generally not detected by this method, because Proteins A and G do not react with IgM molecules. The ReACT method has good specificity and sensitivity comparable to a tube LISS-IAT.

The disadvantages to this technology are similar to those found with the gel test. The efficiency made possible by batch testing is not practical in all laboratory settings. Complex antibody identification usually requires multiple runs of selected cells beyond what is available on the commercially prepared 0.8 percent panel. ABO and D typing and polyspecific and C3d-specific DATs are currently not available by this method. With the ReACT system, it is important to remove test strips from the centrifuge within a few minutes following completion of the centrifugation cycle. This is due to the fixed vertical position of the test strips in the centrifuge. If test strips are not

removed from the centrifuge in a timely fashion after centrifugation, the RBC band can dislodge and disperse through the gel, making test interpretation difficult. This difficulty can easily be avoided by removing the test strips from the centrifuge after the 3-minute cycle and storing them upright until they are read. One last concern is the lack, so far, of licensed automated or semi-automated equipment that can process the ReACT test strips.

Solid-phase technology

The advantages of solid-phase testing are similar to the other two techniques. Compared to tube testing, solid-phase testing requires a smaller sample volume; provides easier handling of large batches, which allows better time management of staff; and is less technique dependent. The most dramatic change, noted by technologists who perform parallel testing, is the greater sensitivity of solid-phase testing compared to the LISS-IAT in tubes.

The disadvantages found with the solid-phase method are quite different from those found with either of the other test methods. There is a learning curve in changing from tube testing to solid-phase testing, which must be completed before the improvement in time management will be achieved. Of interest to the reference laboratories is the fact that this method does not lend itself to RBC membrane modifications such as DTT or AET. Finally, the greater sensitivity, which can be an asset, is also a problem when lack of specificity leads to unnecessary testing. With increased expertise, this concern is manageable.

Instrumentation

The basic pieces of equipment and materials required for the gel test are the dedicated incubator, centrifuge, and RBC diluents. Accessories are also available to make the testing run smoothly. These include specially designed workstations, automatic pipettes with disposable tips, and an automated reader (Reader M, Micro Typing Systems; Ortho). The Reader M instrument utilizes advanced image analysis to digitally read gel cards within seconds. Results can be archived or printed on customized reports.

To handle a larger number of samples, the gel system can include a fully programmable, random-access liquid-handling station (Megaflex-ID, Tecan; Ortho).¹⁵ This instrument will perform the pipetting activities for all routine blood banking tests for the gel system, and it has a 256-sample capacity. The following tests can be

selected from the instrument's test menu: ABO/D typing, ABO reverse typing, Rh phenotyping, antibody detection, antibody identification, crossmatching, and a DAT using anti-IgG only. The gel system offers positive sample and gel card identification, STAT analyses, and computer interface capabilities, and it can process up to 100 two-cell antibody screening tests per hour.¹⁶ The system is semi-automated in that it performs all the pipetting and reading for the user, but someone still has to transfer the gel cards from the pipetting chamber, to the incubator, to the centrifuge, and then to the reader. To take advantage of the instrument's fast pipetting of large volumes of prepared gel cards, more than one set of workstation equipment (incubator and centrifuge) is necessary.

For its implementation as a new manual system, ReACT has a dedicated centrifuge and incubator designed to hold the strip of microcolumns. ReACT system accessories include a viewbox, control cells, and a centrifuge balance kit. At this time, antibody detection, antibody identification, and IgG autocontrol tests can be performed by the ReACT method in the United States. Crossmatching can be performed as long as the facility validates the method for that purpose prior to implementation. Theoretically, even antigen typing could be performed by this method if antigen-specific IgG antibody is first attached to the RBCs via incubation of the RBCs with specific antisera, prior to centrifugation in the ReACT system.

Like the gel and ReACT systems, solid-phase testing can be performed in a manual setting using a custom-designed incubator, centrifuge, and standard plate washer. Unlike the gel and ReACT systems, there is also a fully automated instrument (ABS2000, Immucor) that is a completely integrated, walk-away system that locks once the run is initiated. Access is denied until testing is complete or the run is cancelled. The ABS2000 is a batch analyzer with a menu that includes ABO/D typing, antibody screening (4-cell screen), type and screen, donor confirmations, and the LISS-IAT crossmatch. It was not designed to be a STAT analyzer. For any situation in which crossmatched products must be provided in less than 60 minutes it would be advisable to use the semi-automated equipment for the solid-phase tests. Sandler et al.¹⁷ concluded that the instrument would be useful in hospitals that tested 6000 to 20,000 samples annually. That covers most small to medium-sized hospital transfusion services. It is a fairly compact instrument that sits on a tabletop and requires no special plumbing. Quality control testing of

all product lot numbers in use must be run every 24 hours or the instrument will discontinue testing. All testing and quality control data can be archived or downloaded to another information system.

Discussion

In the consideration of a major change in any system, the goals to be achieved act as the starting point and the standard against which the final outcome will be measured. The goals of automation in most cases include increasing workload capacity by increasing the number of tests performed per full-time equivalent, reducing the number of labor-intensive manual procedures performed, improving precision and accuracy, and eliminating subjectivity of results. All three of the protocols reviewed have been designed to address these issues. However, they do this in different ways. To decide which is the most appropriate system for one laboratory, a comparison of the advantages and disadvantages must be done in the context of the laboratory that will be using the chosen system. First it must be determined if the decision will be made according to choice of test method, or to choice of instrumentation.¹⁸ For example, if the ABS2000 is the instrument of choice, then the laboratory must switch to solid-phase testing.^{19,20}

If the test method is the priority, it can be useful to read existing literature that compares two or more of the new methods. The two factors that should be considered when contemplating this kind of change in serologic testing are (1) eliminating reading after incubation at 37°C,^{21,22} and (2) the factors affecting sensitivity of the DAT and IAT.²³ Over 400 studies have been published comparing gel tests with other methods.²⁴ The results of these studies are highly variable, depending on the method and reagents used to make the comparison. Most reports seem to conclude that the gel test is at least as sensitive if not slightly more so in the detection of clinically significant antibodies as an LISS-IAT performed in a tube. Some reports have shown it to be particularly good at the detection of Rh system antibodies. Two reports comparing the gel test and the PEG-IAT conflict in their conclusions. One report by Derr et al.¹⁸ claimed the gel test is as sensitive or more so than PEG-IAT; the other report in an abstract by Issitt et al.²⁵ claimed PEG-IAT detected clinically significant antibody specificities the gel test did not detect. Another study by Reilly et al.²⁶ compared not only the sensitivity but also the specificity of several IAT methods including LISS and

PEG-IATs. In this study, although PEG had slightly better sensitivity than the other two methods, the gel test had a better balance of both specificity and sensitivity. A study by Weisbach et al.²⁷ compared the gel test, ReACT, and two other column agglutination systems available in Europe to the LISS-IAT in tubes. They found that the gel test and ReACT had very similar rates of antibody detection (91.6 % and 90.3%, respectively) and also the lowest detection rates of clinically insignificant antibodies (28% and 39%, respectively) of the four column systems. One study, reported by Shirey et al.²⁸ compared ReACT to LISS-IAT in tubes. ReACT technology was nonreactive with all 130 LISS-IAT negative samples, yielding a 0 percent false positivity. On the other hand, all 84 or 100 percent of the clinically important antibodies were detected by ReACT, as they had been by LISS-IAT.

Long-term cost containment is often cited as one of the reasons to implement automation. Choosing a test method should include consideration of types of antibodies that must be detected by the system versus antibodies that are not considered clinically significant in a laboratory. This frame of reference will shift according to the laboratory involved. For example, transfusion services concentrate on finding compatible blood products, whereas reference laboratories need to identify all antibodies before recommending the safest option for transfusion. The literature contains reports showing how well the techniques in this review detect antibody specificities or how efficiently they miss some specificities, depending on the perspective of the author. For example, Shirey et al.²⁸ found that five (31%) of the 16 tested samples containing clinically benign specificities, such as anti-Sd^a, cold auto agglutinins, and Lewis and Knops system antibodies, were nonreactive by ReACT technology, which is an IgG-specific test; Rolih et al.²⁹ found that antibodies to antigens such as Le^a, Le^b, M, N, and P₁ were detected by solid-phase only if there was IgG class antibody present. Rolih et al. were also able to exclude crosslinking of IgM-class antibodies between the monolayer and antigen-positive indicator RBCs as a contributor to the detection of "clinically insignificant" antibodies; in the process, they found that 28 (48%) of 59 insignificant antibodies tested did not react by solid phase. In a cost analysis, Chan et al.³⁰ reported that the gel test missed 11 (31%) of 36 "cold antibodies" that had been detected by LISS-IAT. Thus, a decrease in detection of unwanted specificities in the range of 30 to 50 percent can be anticipated by

transfusion services before any on-site evaluation is performed.

A comparison of the instrumentation available will involve a work-flow analysis to find the best fit. Each laboratory has its own ways of serving its customers. If the workload requires a large amount of component preparation (platelet pooling) and issuing of components to patients, a system that offers walk-away testing and frees technologists to perform these tasks is preferred. If large amounts of RBCs are transfused and fewer plasma components prepared, a system for faster serologic testing to decrease the number of units released before antibody screening is complete would be the system of choice.

With the decrease in budgets, loss of specialized staff, and increased regulatory requirements, there is no longer the option of choosing the test of record and any accompanying equipment by the old yardstick of popularity with the staff. The educated consumer will prepare a list of requirements, compare all the variables within his or her own laboratory, and choose the system that offers the most improvement over the previous method.

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Comparison of tube and gel techniques for antibody identification

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There are several methods for antibody detection and each technique has advantages and limitations. We compared the performance of the tube (polyethylene glycol-indirect antiglobulin test [PEG-IAT]) and gel test technique for antibody identification. From January to May 1999, we performed antibody screening tests by gel and tube techniques on 10,123 random blood samples submitted to our reference laboratory. Six hundred and twenty-eight (6.2%) reactive samples were tested for antibody specificity by both methods. One hundred and ninety-six were reactive only by gel: 25 anti-D, 33 anti-C, 76 anti-E, 13 anti-c, 5 anti-e, 18 anti-K, 7 anti-Jk^a, 2 anti-Di^a, 3 anti-S, 8 combination Rh antibodies (1 with anti-K), and 6 other antibody specificities. Two samples were reactive only by PEG-IAT: 1 anti-K and 1 anti-Di^a. Four hundred and thirty were positive by the two methods: 156 anti-D, 9 anti-C, 68 anti-E, 15 anti-c, 6 anti-e, 61 anti-K, 12 anti-Jk^a, 17 anti-Di^a, 5 anti-S, 73 combination Rh antibodies (2 with anti-K), and 8 other antibody specificities. Based on this study, the gel test is more sensitive ($p < .01$) than the tube test for identifying potentially clinically significant antibodies. *Immunohematology* 2000; 16:138-141.

Red blood cell diluent composition is important for detection of some anti-E

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Commercially prepared 0.8% reagent red blood cells (RBCs) eliminate the need to manually dilute 3 to 5% RBCs for use in gel cards. Ortho-Clinical Diagnostics investigated twelve anti-E samples detected in MTS Anti-IgG gel cards using Ortho 3% reagent RBCs manually diluted to 0.8% in MTS Diluent 2TM (MTS2) that were not detected with commercially prepared Ortho 0.8% reagent RBCs. In gel tests, using additional examples of E-positive RBCs, 22 of 26 anti-E were reactive when the cells were suspended in MTS2. Only 6 of 28 anti-E were reactive with E-positive Ortho 0.8% reagent RBCs. Five anti-E were tested in gel with five R₂R₂ RBCs that had been washed and resuspended in four low-ionic-strength diluents. Fifty-eight percent of tests performed in MTS2 were positive compared to 13 to 41 percent for the other diluents. Anti-E detection also varied from 6 to 56 percent based on the donor of the RBCs. Seven anti-E were characterized by their reactivity in tube techniques and were reactive using PEG and/or ficin-treated RBCs only. As a comparison, 25 archived examples of anti-E were detected using RBCs suspended in MTS2 and Ortho 0.8% reagent RBCs. These data show that some anti-E are not detected by Ortho reagent RBCs in MTS Anti-IgG gel cards. However, these anti-E have characteristics of antibodies of questionable clinical significance. *Immunohematology* 2000; 16:142-146.

Quantitation of red cell-bound immunoglobulins and complement in lymphoma patients

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Quantitative ELISA may be useful for determining the amount of red blood cell (RBC)-associated immunoglobulins (Igs) in patients with autoimmune hemolytic anemia (AIHA). In idiopathic AIHA, there is about 20 times more RBC-associated IgG and complement than in normal persons. In patients with low-grade lymphomas (particularly, B-CLL and splenic marginal zone lymphoma) autoimmune hemolysis is a component of their anemia. In high-grade malignant lymphomas (i.e., diffuse large B-cell lymphoma and peripheral T-cell lymphoma), as well as in Hodgkin's disease, autoimmune hemolysis contributes little, if any, anemia. The quantitative ELISA for RBC-associated IgG and complement is useful for following the effects of treatment in patients with immune hemolysis. *Immunohematology* 2000; 16:147–153.

A quick and simple method for phenotyping IgG-sensitized red blood cells

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Positive (IgG) direct antiglobulin test (DAT) reactivity ranging from weakly positive to 2+ can be eliminated using a simple “blocking” technique with anti-IgG. This method can be used for antigen typing DAT-positive red blood cells that require the antiglobulin technique. *Immunohematology* 2000;16:154–156.

Further characterization of transfusion-related acute lung injury: demographics, clinical and laboratory features, and morbidity

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According to Food and Drug Administration data, transfusion-related acute lung injury (TRALI) is the third most frequent cause of transfusion-associated death in the United States and is characterized by an acute respiratory distress syndrome-like clinical picture following transfusion of plasma-containing blood components. It may be underdiagnosed due to unfamiliarity of clinicians with the syndrome. This report describes the largest series to date, 46 cases, occurring between 1992 and 1998. The male-to-female ratio was approximately 1:1. The mean age at diagnosis was 54 years. The most frequent presenting symptom or signs were acute respiratory distress, hypotension, and hypertension. Antibodies to human leukocyte antigens or granulocytes were identified in 61 percent of cases, with 50 percent associated with antibodies in a donor whose blood had been transfused to a patient developing TRALI. Clinical recovery occurred in 87 percent of patients, but TRALI contributed to deaths in 13 percent. Clinicians need to recognize and diagnose this syndrome in order to respond with appropriate interventions. *Immunohematology* 2000;16:157–159.