

Immunohematology



Journal of Blood Group Serology and Molecular Genetics

VOLUME 30, NUMBER 3, 2014



**American
Red Cross**

Immunohematology

Journal of Blood Group Serology and Molecular Genetics

Volume 30, Number 3, 2014

CONTENTS

113	SEROLOGIC METHOD REVIEW Cold acid elution (ELU Kit II) M. Hinrichs and M.A. Keith
117	EDUCATIONAL FORUM CASE REPORT A case of masquerading alloantibodies: the value of a multitechnique approach P.M.S. Wennersten and L.J. Sutor
121	BLOOD GROUP ALLELE REPORT <i>RHCE</i> variant allele: <i>RHCE</i>*ce254G,733G J.A. Keller, T. Horn, C. Chiappa, C. Melland, C. Vietz, L. Castilho, and M.A. Keller
123	ORIGINAL REPORT Detection and identification of platelet-associated alloantibodies by a solid-phase modified antigen capture enzyme-linked immunosorbent assay method and its correlation to platelet refractoriness in multiplatelet concentrate–transfused patients N. Jain, R.S. Sarkar, and J. Philip
126	ORIGINAL REPORT Effects of pH changes of stock normal saline solution on 5 percent red cell suspension G.L. Martin, P.J.M. Caraan, J.J.S. Chua, J.A.L. Crescini, J.M.C. Diokno, C.B.Dlr. Javier, M.K.B.O. Reyes, and R.Y. Soliven
135	COMMUNICATION Unusual erythrocyte split chimerism in pregnancy after allogeneic stem cell transplantation M.L. Barjas-Castro, A.C. Vigoritto, F.A. Moretto, and V. Castro
137	ANNOUNCEMENTS
141	ADVERTISEMENTS
145	INSTRUCTIONS FOR AUTHORS

EDITOR-IN-CHIEF

Sandra Nance, MS, MT(ASCP)SBB
Philadelphia, Pennsylvania

MANAGING EDITOR

Cynthia Flickinger, MT(ASCP)SBB
Philadelphia, Pennsylvania

TECHNICAL EDITORS

Christine Lomas-Francis, MSc
New York City, New York

Joyce Poole, FIBMS
Bristol, United Kingdom

Dawn M. Rumsey, ART(CSMLT)
Norcross, Georgia

SENIOR MEDICAL EDITOR

Ralph R. Vassallo, MD
Philadelphia, Pennsylvania

ASSOCIATE MEDICAL EDITORS

P. Dayand Borge, MD
Baltimore, Maryland

David Moolten, MD
Philadelphia, Pennsylvania

MOLECULAR EDITOR

Margaret A. Keller
Philadelphia, Pennsylvania

EDITORIAL ASSISTANT

Sheetal Patel

PRODUCTION ASSISTANT

Marge Manigly

COPY EDITOR

Mary L. Tod

PROOFREADER

Wendy Martin-Shuma

ELECTRONIC PUBLISHER

Paul Duquette

EDITORIAL BOARD

Patricia Arndt, MT(ASCP)SBB
Pomona, California

Barbara J. Bryant, MD
Milwaukee, Wisconsin

Lilian Castilho, PhD
Campinas, Brazil

Martha R. Combs, MT(ASCP)SBB
Durham, North Carolina

Geoffrey Daniels, PhD
Bristol, United Kingdom

Anne F. Eder, MD
Washington, District of Columbia

Melissa R. George, DO, FCAP
Hershey, Pennsylvania

Brenda J. Grossman, MD
St. Louis, Missouri

Christine Lomas-Francis, MSc
New York City, New York

Geralyn M. Meny, MD
San Antonio, Texas

Paul M. Ness, MD
Baltimore, Maryland

Thierry Peyrard, PharmD, PhD
Paris, France

Mark Popovsky, MD
Braintree, Massachusetts

S. Gerald Sandler, MD
Washington, District of Columbia

Jill R. Storry, PhD
Lund, Sweden

David F. Stroncek, MD
Bethesda, Maryland

Nicole Thornton
Bristol, United Kingdom

EMERITUS EDITORS

Delores Mallory, MT(ASCP) SBB
Supply, North Carolina

Marion E. Reid, PhD, FIBMS
New York City, New York

Immunohematology is published quarterly (March, June, September, and December) by the American Red Cross, National Headquarters, Washington, DC 20006.

Immunohematology is indexed and included in *Index Medicus* and MEDLINE on the MEDLARS system. The contents are also cited in the EBASE/Excerpta Medica and Elsevier BIOBASE/Current Awareness in Biological Sciences (CABS) databases.

The subscription price is \$50 for individual, \$100 for institution (U.S.) and \$60 for individual, \$100 for institution (foreign) per year.

Subscriptions, Change of Address, and Extra Copies:

Immunohematology, P.O. Box 40325
Philadelphia, PA 19106

Or call (215) 451-4902

Web site: www.redcross.org/about-us/publications/immunohematology

Copyright 2014 by The American National Red Cross
ISSN 0894-203X

ON OUR COVER

Konstantin Somov was a Russian symbolist painter associated with *Mir iskusstva* (World Of Art) in Russia, both sobriquet for a *Fin de siècle* movement and magazine devoted to novel artistic vision and methods that influenced the overall evolution of modernism in Europe. Proponents rejected positivist or revivalist influences and preferred watercolor and gouache to oil. They also often worked closely with both the theater and the ballet. Somov had a special fondness for the harlequin motif in his painting, tending to find an inherent theatricality to life, and a number of his works use the masquerade as a central theme. This work, titled *Masquerade*, evinces his style and also alludes to Wennersten and Sutor's case of masquerading alloantibodies, which appears in this issue.

DAVID MOOLTEN, MD



Cold acid elution (ELU Kit II)

M. Hinrichs and M.A. Keith

Elution is a procedure for recovery of antibody attached to intact, immunoglobulin-coated red blood cells (RBCs) by disrupting the antigen–antibody bonds. The recovered antibody is collected in an inert diluent and is referred to as an eluate. Testing of an eluate may be desired to identify antibody(ies) coating the RBCs of patients with a positive direct antiglobulin test. Many types of elution procedures have been developed and described; however, an acid elution is suitable for antibody recovery in most cases, such as recovery of alloantibodies and warm-reactive autoantibodies. Studies have compared methods such as xylene, chloroform, digitonin acid, dichloromethane, citric acid, and Immucor Elu-Kit II (cold acid elution). The ELU-Kit II has been shown to be quick and effective at eluting a wide range of alloantibodies as well as autoantibodies without the use of hazardous chemicals or costly reagent preparation time that some methods use. It is for these reasons that the ELU-Kit II is a very popular method for the elution of immunoglobulin G (IgG) antibodies. *Immunohematology* 2014;30:113–116.

Key Words: antibody recovery, acid elution, IgG-coated RBCs

Elution is a procedure for recovery of antibody attached to intact, immunoglobulin-coated red blood cells (RBCs) by disrupting the antigen–antibody bonds.¹ The recovered antibody is collected in an inert diluent and is referred to as an eluate.² Testing of an eluate may be desired to identify antibody(ies) coating the RBCs of patients with a positive direct antiglobulin test (DAT).³ Many types of elution procedures have been developed and described; however, an acid elution is suitable for antibody recovery in most cases, such as recovery of alloantibodies and warm-reactive autoantibodies.⁴ Studies have compared methods such as xylene, chloroform, digitonin-acid, dichloromethane, citric acid, and Immucor Elu-Kit II (cold acid elution).^{1,3,5} The ELU-Kit II has been shown to be quick and effective at eluting a wide range of alloantibodies as well as autoantibodies without the use of hazardous chemicals or costly reagent preparation time that some methods use.⁵ It is for these reasons that the ELU-Kit II is a very popular method for the elution of immunoglobulin G (IgG) antibodies.

Principle

For an antibody to be effectively eluted from RBCs, the antigen–antibody complexes must be disturbed by reversing or neutralizing the binding forces. This can be accomplished by altering the pH of the solution.¹

Reagents/Supplies

Reagents	Supplies
<ul style="list-style-type: none">Concentrated wash solutionEluting solutionBuffering solutionIsotonic saline or phosphate-buffered isotonic saline (pH 6.5–7.5)Anti-human globulin containing anti-IgGIgG-sensitized red blood cellsPolyethylene glycol additive (optional)	<ul style="list-style-type: none">12- × 75-mm test tubesStopper/caps for tubesPipettes (glass or plastic)Centrifuge37°C incubator or water bathTimerOptical aid

Procedural Steps

Preparing the Eluate

- Wash RBCs one time with saline and four times with working wash solution
- Reserve an aliquot of the last wash supernatant for use as a control in testing
- Place 1 mL (approximately 20 drops) of washed RBCs in a clean, labeled test tube
- Add an equal amount of eluting solution
- Cap the tube and gently invert four times
- Immediately centrifuge 45–60 seconds
- Transfer supernatant to a clean, labeled tube
- Add sufficient buffering solution to restore the pH of the eluate (indicated by a pale blue color)
- Mix well and centrifuge

Testing the Eluate

- Add 1 drop washed RBC suspension or commercial reagent RBC to labeled test tube
- Add a small volume of saline, mix, and centrifuge
- Decant saline and blot dry
- Add 2 drops of eluate, mix, and incubate 10–30 minutes at 37°C
- Wash cells one time with working wash solution
- Centrifuge, decant working wash solution, and blot dry
- Add anti-IgG, mix, centrifuge, and read

RBC = red blood cell.

RBCs coated with IgG antibody are first thoroughly washed with saline or a buffered wash solution to remove all unbound protein while maintaining the associated bound antibody without lysing the RBCs. The washed RBCs are suspended in a low pH glycine-HCl solution to dissociate the bound antibody. After centrifugation to separate the RBCs from

the dissociated antibody, the antibody-containing supernatant is then neutralized back to a normal pH with a buffering solution. The remaining eluate may then be tested by a method appropriate for detection of IgG antibodies.¹ The eluate should be tested in parallel with an aliquot of supernatant from the final wash of the RBCs to ensure that antibody detected in the eluate is RBC-bound antibody and not residual plasma antibody.⁴

Indications

Eluates may be prepared and tested to identify antibody(ies) on the RBCs of patients with a positive DAT caused by autoantibodies or alloantibodies. Elution studies are an important component to the laboratory diagnosis of immune-mediated RBC destruction caused by alloantibodies, autoantibodies, and drug-induced phenomena. In patients who have received RBC transfusion in the previous 3 to 4 weeks and develop a positive DAT, eluate testing can be very important in identifying newly developing antibodies or delayed hemolytic transfusion reactions.⁶ Eluates can be prepared from RBCs coated with antibody in vivo or in vitro. When used in combination with in vitro adsorption techniques, elutions may also be desired to confirm the presence or absence of a weakly expressed antigen on RBCs, to isolate a single antibody specificity from a plasma containing multiple alloantibodies, or to concentrate antibody-containing solutions.¹

Procedure

Preparing the Eluate¹

RBCs should be washed thoroughly until the supernatant is free of hemolysis using saline or working wash solution (ELU-Kit II). If the working wash solution is to be used, wash the RBCs one time with saline followed by four washes with the working wash solution. It is recommended that the washing be performed using saline or working wash solution chilled to 1° to 10°C to minimize the loss of bound antibody. Reserve an aliquot of the last wash supernatant for use as a control in testing.

Place 1 mL (or approximately 20 drops) of washed RBCs in a clean 12- × 75-mm test tube. If there is an insufficient amount of RBCs to yield 1 mL of cells, a smaller volume of RBCs may be used. Add an equal amount of eluting solution to the tube containing the RBCs, cap, and gently invert the tube four times. To prevent hemolysis, immediately centrifuge the tube 45 to 60 seconds, and transfer the supernatant to a clean tube.

To the tube containing the acidic eluate, add 1 mL of buffering solution or an amount equal to the volume of RBCs used for eluting. As the pH is restored to the range acceptable for testing (6.4 to 7.6), the color of the solution should change from yellow to a pale blue. If the eluate remains yellow after 20 drops of buffering solution has been added, continue to add buffering solution dropwise until a pale blue color persists. Mix well and centrifuge to remove any precipitate that formed during neutralization, and transfer the supernatant to a clean, properly labeled tube. The eluate may now be tested by a desired indirect antiglobulin method for antibody identification.

Testing the Eluate

Potentiators such as bovine albumin or low-ionic-strength saline are not usually necessary because the eluate has been prepared in a low-ionic substrate. If the use of polyethylene glycol (PEG) additive is desired to enhance the antibody reactivity, the modified antiglobulin test should not be used.¹ Some studies have shown that eluates can be effectively tested using gel microcolumn assay or solid-phase red cell adherence assay⁷; however, these methods should be validated by individual laboratories before routine use.

Add one drop of washed RBC suspension or commercial reagent RBCs to a labeled test tube. Add a small volume of saline (5–10 drops), mix thoroughly, and centrifuge 30 seconds. Decant the saline supernatant and blot the tube dry.¹

Add two drops of the eluate to the dry cell button, mix thoroughly, and incubate at 37°C ± 1°C for 10 to 30 minutes. If PEG enhancement is added, follow the manufacturer's instructions for incubation time.¹

After incubation, add 5 to 10 drops of working wash solution to the tube, mix thoroughly, and centrifuge for 30 seconds. Decant the working wash solution supernatant and blot the tube dry. This method is referred to as the modified antiglobulin test. If PEG was used in the test system, the modified antiglobulin test should not be performed; instead, the RBCs should be washed at least three times with saline before adding anti-IgG. It is not necessary to wash the tube containing PEG using the working wash solution. Add anti-IgG as directed to the dry cell button, mix, and centrifuge. Examine the tube for agglutination and record results. IgG-sensitized RBCs should be added to all negative antiglobulin tests to confirm anti-IgG was added to the test system and to thus validate the test results.¹

The last wash supernatant should be tested for antibody reactivity in parallel with the eluate to ensure that antibody identified in the eluate is not a result of inadequate washing of

the RBCs.¹ There should be no antibody reactivity detected in the last wash control tubes.

Quality Control

An aliquot of the last wash supernatant should be retained and tested in parallel with the eluate. Negative results should be obtained in the last wash control testing to demonstrate that the reactivity observed in the eluate is not unbound antibody in suspension as a result of inadequate washing of the red blood cells before the elution process. If reactivity is observed with the last wash control, the elution procedure should be repeated after more thorough washing of the RBCs.^{1,8}

Limitations

If antibody reactivity is detected in the last wash control, the eluate results should be considered invalid and all steps should be repeated. Antibody reactivity detected in the last wash may be caused by inadequate washing of the RBCs or may be owing to dissociation of the antibody from the RBCs during the washing process. To minimize the amount of antibody dissociation that occurs during washing of the RBCs, washing the RBCs with cold (1–10°C) working wash solution before elution may be beneficial.¹

A nonreactive eluate prepared from RBCs demonstrating a positive DAT can indicate the presence of drug-dependent antibodies, nonimmunologic uptake of IgG onto the RBCs, or possibly an antibody to a low-prevalence antigen not present on panel cells tested.⁶

RBCs that have been used for this elution process must be discarded and should not be used for testing, such as antigen typing or autoadsorption. Acid elution procedures do not significantly reduce the strength of the DAT, which renders the RBCs unsuitable for phenotyping. If antigen typing or autoadsorption is desired, another elution method (such as one that uses citric acid) should be used.³

RBCs that have been stored for longer than 72 hours may produce less potent eluates when compared with eluates prepared from freshly drawn samples. A different elution method may be more effective for preparing an eluate from RBCs with prolonged storage.¹

Hemolysis of RBCs may occur if there is prolonged contact with the eluting solution. The RBCs must be centrifuged immediately after addition of the eluting solution and the supernatant transferred to a clean test tube.¹

Hemolysis of RBCs may also occur if the pH of the eluate is not restored to neutral pH range before testing. This may result in false-negative results.¹

False-positive results may occur with the use of the working wash solution as a result of the presence of a nonspecific high-titer antibody. In this case, saline should be used as a substitute for washing the RBCs before the addition of eluting solution.¹

False-negative results may occur with use of the modified antiglobulin test if the test RBC suspension is not thoroughly washed to remove all human protein. Use of IgG-sensitized RBCs will assure that this has not occurred if a positive result is obtained after addition of the IgG-sensitized RBCs. A negative result after addition of IgG-sensitized RBCs should alert the tester to this possible source of error.¹

Consideration may be given to selecting an elution method that yields a more potent eluate than other methods for certain antibody specificities. For example, the ELU-Kit II method yields more demonstrable anti-e than the chloroform, digitonin acid, or dichloromethane methods. However, the ELU-Kit II method has been shown to be less effective at recovering anti-Jk^b than other methods.⁵

Performance of antibody elutions as a result of a positive DAT caused by IgG should be reserved for those patients who have been recently transfused or are suspected of having immune-mediated hemolysis. Studies have shown that the routine performance of antibody elutions on all patients with a positive DAT is not clinically useful because the majority of positive DATs yield negative eluates and, of those that yield positive reactivity, the majority of the eluted antibodies are also demonstrable in the serum.^{9,10}

Summary

The Gamma ELU-Kit II is an effective method for the rapid acid elution of antibodies from intact RBCs. However, there is no single elution method that is ideal for every situation. If an eluate prepared by cold acid elution (ELU Kit II) gives inconclusive results, it may be beneficial to prepare an eluate by a different method.

References

1. Immucor, Inc. Gamma ELU-Kit™ II manufacturers insert. Insert code: IC3021-2. Revised September 2012.
2. Issitt P, Anstee D. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998: 56–58.
3. Burich M, AuBuchon J. Evaluation of a rapid technique for antibody elution. *Immunohematology* 1986;2:74–6.
4. Roback JD. Technical manual. 17th ed. Method 4-1, cold-acid elution procedure. Bethesda, MD: AABB, 2011;917–18.
5. South S, Rea A, Tregellas W. An evaluation of 11 red cell elution procedures. *Transfusion* 1986;26:167–70.
6. Yazer M, Triulzi D. The role of the elution in antibody investigations. *Transfusion* 2009;49:2395–9.
7. Finck R, Davis R, Teng S, et al. Performance of an automated solid-phase red cell adherence system compared with that of a manual gel microcolumn assay for the identification of antibodies eluted from red blood cells. *Immunohematology* 2011;27:1–5.
8. Judd WJ, Johnson ST, Storry JR, eds. Judd's methods in immunohematology. 3rd ed. IV-E. Eluting antibodies with cold acid. Bethesda, MD: AABB, 2008;128–31.
9. Domen R, Grattan J. Efficacy of performing red-cell antibody elutions in patients with a positive direct antiglobulin test. *Vox Sang* 1986;51:324–6.
10. Stec N, Shirey RS, Smith, B, Kickler TS, Ness PM. The efficacy of performing red cell elution studies in the pretransfusion testing of patients with positive direct antiglobulin tests. *Transfusion* 1986;26:225–6.

Monica Hinrichs, MT (ASCP), Lead Technologist, Immunohematology Reference Lab, American Red Cross, Mid-America Blood Services Division; Monica A. Keith, MS, BB (ASCP), (corresponding author), Manager, Immunohematology Reference Lab, American Red Cross, Mid-America Blood Services Division, 405 W. John Gwynn Jr. Ave., Peoria, IL 61605-2440.

Important Notice About Manuscripts for *Immunohematology*

Please e-mail all manuscripts to immuno@redcross.org

Notice to Readers

Immunohematology is printed on acid-free paper.

Attention: SBB and BB Students

You are eligible for a **free** 1-year subscription to *Immunohematology*.

Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to immuno@redcross.org

For information concerning *Immunohematology* or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@redcross.org

A case of masquerading alloantibodies: the value of a multitechnique approach

P.M.S. Wennersten and L.J. Sutor

In an immunohematology reference laboratory, samples received for antibody identification react in many different ways requiring a variety of approaches. Sometimes, the clues from initial testing can lead to faulty assumptions and misdirection. Fortunately, a well-supplied reference laboratory will have access to a variety of techniques and reagents that, when used together, can reveal the true identity of the antibodies involved. We present a case of a patient sample with an apparent group AB, D+ blood type showing strong reactivity with all cells tested in the forward and reverse ABO, in the D testing as well as in a three-cell antibody screen. The initial assumption was that the plasma contained a cold autoantibody. Subsequent testing, including the use of gel column technology, ficin-treated cells, and antisera for phenotyping, showed the apparent cold autoantibody to be a red herring. Additional tube testing at immediate spin, 37°C, and indirect antiglobulin test (IAT) revealed the presence of four alloantibodies: anti-M and anti-E reacting at immediate spin, 37°C, and IAT plus anti-Fy^a and anti-Jk^b reacting at IAT. *Immunohematology* 2014;30:117–120.

Key Words: ABO discrepancy, cold autoantibodies, gel column technology, ficin, alloantibodies

Clinical Case Presentation

A sample from a 65-year-old man was received in our immunohematology reference laboratory for antibody identification and crossmatch. The referring hospital reported that his antibody screen had been negative by a solid-phase technique 3 weeks prior, and 1 unit of red blood cells (RBCs) had been transfused at that time. The sample sent to our laboratory was tested at the referring facility by an automated solid-phase method (Galileo Echo, ImmucorGamma, Norcross, GA) with all three-screen cells strongly reactive (4+) and an undetermined blood type.

Immunohematologic Evaluation and Results

Testing was initiated using tube techniques for ABO and D typing, direct antiglobulin test (DAT), and antibody screen. The initial testing showed strong reactivity with all cells tested in the forward and reverse ABO typing as well as in the D testing and in a three-cell antibody screen (Tables 1 and 2). The antibody three-cell screen (ALBAcyte, Quotient,

Table 1. Initial ABO and D typing

Forward		Reverse		Anti-D
Anti-A	Anti-B	A1 Cells	B Cells	
4+	4+	4+	4+	4+

Table 2. Antibody screen by tube methods

Cell	LISS 37°C	LISS IAT	PEG IAT
I	4+	4+	4+
II	4+	4+	4+
III	4+	4+	4+
Auto	0	0√	0√

LISS = low-ionic-strength saline; IAT = indirect antiglobulin test; PEG = polyethylene glycol.

Newtown, PA) was tested at 37°C using low-ionic-strength saline (LISS) and by the indirect antiglobulin test (IAT) using LISS and polyethylene glycol (PEG). At this point, it was unclear whether the ABO discrepancy was because of a problem with the patient's cells or plasma, or both. Because the 6 percent albumin control and DAT with polyspecific anti-human globulin were negative, unexpected plasma reactivity was suspected. A cold antibody screen was performed, with all three-screen cells strongly reactive (4+) and the auto control weakly reactive (1+w) at immediate spin (IS). Review of the antigen profile sheet for these screen cells made it clear that these reactions were not caused by one of the usual cold-reactive suspects (anti-M, anti-P1, anti-Le^a, or anti-Le^b) acting alone (Table 3).

Even though the auto control was negative by IAT, the possibility of a cold autoantibody with anti-IH specificity was supported by the strong reactivity of the auto control at 4°C and the patient's apparent AB blood group. The patient was typed for the A1 antigen and found to be positive, further supporting the possibility of a cold-reactive autoanti-IH. Patient RBCs were nonreactive with anti-H lectin, as would be expected for cells with the A1B blood group.¹ Additional testing was performed with group A, B, and O cord blood cells as well as panel cells negative for the I antigen. All were strongly reactive at IS. The antibody screen was also repeated with an LISS prewarming technique and a saline no-enhancement technique, but strong reactions (4+) were still observed at 37°C and by the IAT.

Table 3. Cold antibody screen

Cell	D	C	E	c	e	f	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P1	M	N	S	s	IS	RT	4C
I	+	+	0	0	+	0	0	+	0	+	+	0	0	+	0	+	+	0	+	4+	4+	4+
II	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	4+	4+	4+
III	0	0	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	+	0	4+	4+	4+
Auto control																				1+w	1+	4+

IS = immediate spin; RT = room temperature.

Table 4. Antibody screen by the IgG gel test

Cell	D	C	E	c	e	f	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P1	M	N	S	s	Gel
1	+	+	0	0	+	0	0	+	0	+	+	+	+	0	+	+	0	+	+	4+
2	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	4+
3	0	0	0	+	+	+	+	+	+	0	+	0	0	+	S	+	+	+	+	4+
4	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	+	0	+	0	4+
5	+	0	+	+	0	0	0	+	+	0	0	+	0	+	+	+	+	0	+	4+
6	0	0	0	+	+	+	+	+	+	0	+	0	+	0	+	0	+	+	+	3+

Table 5. Antibody identification panel using the IgG gel test with untreated and ficin treated panel cells

Cell	D	C	E	c	e	f	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P1	M	N	S	s	Gel	Ficin gel*
1	0	0	+	+	+	+	0	+	0	0	+	+	0	0	+	0	+	0	+	3+	
2	0	0	0	+	+	+	0	+	0	+	+	0	0	+	S	0	+	0	+	0	
3	+	0	+	+	0	0	0	+	+	0	+	0	0	+	S	0	+	+	+	4+	
4	+	+	0	+	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	2+	
5	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	+	3+	0
6	+	0	0	+	+	+	0	+	+	0	+	+	0	0	+	0	+	0	+	3+	3+s
7	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	0	+	0	+	2+s	0
8	0	0	0	+	+	+	0	+	+	0	+	0	+	0	0	0	+	0	+	2+s	0
9	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	0	+	0	+	2+	3+
10	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	
11	+	0	0	+	+	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	
12	0	0	0	+	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	

s = strong.

*Per the manufacturer, the use of IgG gel cards (instead of buffer cards) when testing ficin-treated cells is acceptable as long as validation is performed.

Because we needed enough plasma to resolve the ABO discrepancy as well as explain reactions at IS, 37°C, and IAT, the decision was made to try gel testing (ID-MTS Gel Test, Ortho Clinical Diagnostics, Rochester, NY) using screen cells from two different manufacturers (Surgiscreen, Ortho Clinical Diagnostics; Panoscreen, ImmucorGamma). Although this was attempted primarily to save limited sample, it provided the first valuable clue: the one cell reacting weaker than the rest was negative for the M antigen (Table 4). Based on this clue, the patient was typed for the M antigen and found to be M-. The ABO plasma typing (reverse) was repeated with prewarmed plasma and M- group A and B cells in parallel with the screen cells; both M- cells were nonreactive, whereas

the prewarmed screen cells remained positive, showing the apparent cold autoantibody to be a red herring. Although this resolved the ABO discrepancy, it did not explain the reactions with M- cells at all phases of testing, including IS, so an M- selected cell panel was tested in gel using both untreated and ficin-treated cells (0.8% Resolve Panels A, B, and C; Ortho Clinical Diagnostics) tested in parallel with IgG gel cards (Table 5). Finally, some negative reactions were observed.

The ficin-treated cells tested in gel provided the most useful clue, since three of five cells that reacted when untreated were nonreactive when treated with ficin and two reactions were stronger with ficin-treated cells, pointing toward at least one antibody directed at a ficin-sensitive antigen and one

Table 6. Patient phenotype

D	C	E	c	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P1	M	N	S	s
+	+	0	+	0	0	+	+	0	+	0	0	0	+	0	+

fortunate that these cells were also apparently negative for the E antigen, as the anti-E was reactive at the IS phase of testing and was responsible for the unexpected reactivity seen with the M– screen cells tested in the cold antibody screen. Had one

Table 7. Additional antibody identification panel by prewarmed tube methods*

Cell	D	C	E	c	e	f	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P1	M	N	S	s	IS	LISS 37°C	LISS IAT	PEG IAT
1	+	0	+	+	0	0	0	+	0	0	+	0	0	+	+	0	+	0	+	3+	4+	4+	4+
2	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	0	0	+	4+	4+	4+	4+
3	0	0	0	+	+	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	0	2+	2+s
4	0	0	0	+	+	+	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0	0√	0√
5	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	0	2+	2+s
6	0	0	0	+	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	NT	0	0√	0√
7	+	+	0	0	+	0	+	+	0	+	+	0	0	+	+	0	+	0	+	NT	0	0√	0√
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	+	0	+	0	0	0√	0√

*This is a partial panel and does not include all cells tested.

IS = immediate spin; LISS = low-ionic-strength saline; IAT = indirect antiglobulin test; PEG = polyethylene glycol.

antibody with enhanced reactivity to a ficin-resistant antigen in addition to anti-M. At this point, a complete phenotype was performed on the patient's cells (Table 6). Despite the recent transfusion (1 unit of RBCs 3 weeks prior), no mixed field reactivity was observed with neat patient cells. A selected cell panel based on these results was tested by prewarmed tube techniques at IS, LISS 37°C, LISS IAT, and PEG IAT (Table 7). This additional tube testing revealed the presence of four alloantibodies: anti-M and anti-E reacting at IS, LISS 37°C, LISS IAT, and PEG IAT plus anti-Fy^a and anti-Jk^b reacting at LISS and PEG IAT.

One unit of RBCs negative for the M, E, Fy^a, and Jk^b antigens was found to be compatible by LISS IAT crossmatch and was transfused without incident. No further samples have been received from this patient at our facility or at the referring hospital.

Interpretation

Although this case initially presented with characteristics common to cold autoantibodies, all of the unexplained reactions, including the discrepant ABO typing results, were found to be caused by a combination of four alloantibodies including two antibodies reactive at the IS phase of testing. The strongly reactive auto control tested at 4°C may indicate the presence of a cold autoantibody, but this autoantibody was not responsible for the strong reactivity with reagent cells observed at IS, 37°C, and IAT phases of testing. We were able to prove that the patient's blood group was AB by using cells negative for the M antigen for the ABO reverse typing. We were

or both of these cells been positive for the E antigen, the ABO discrepancy would not have been resolved, reactions may have pointed toward the wrong blood type, and the antibody identification would have continued to elude us.

The supplemental use of a gel technique allowed for the identification of these four alloantibodies, reactive at multiple phases of testing, without exhausting the limited amount of sample received. The addition of ficin-treated cells provided beneficial clues and further facilitated the antibody identification process while consuming very little additional sample. The use of patient phenotyping also allowed us to focus our testing based on antigens for which the patient tested negative, and by extension, alloantibodies he could make. Without this flexibility in testing methodology, we most likely would have exhausted the plasma without resolving the antibody identification, resulting in a delay in transfusion.

Discussion

Cold autoantibodies are a frequent cause of both ABO discrepancies and unexpected reactivity with antibody screening cells. Cold-reactive alloantibodies, most often anti-M, anti-P1, anti-Le^a, or anti-Le^b, are also commonly identified as the cause of these reactions.² When unexpected reactions are found at the IS and 37°C phases of testing, it is easy to assume that either cold autoantibodies or "typical" cold-reactive alloantibodies are the cause. However, it is important to remember that many antibodies can react at these phases of testing if they are IgM, which is to be expected for newly

formed alloantibodies. An accurate transfusion history can prove invaluable in such situations.

The resolution of discrepant ABO typing results involves a variety of approaches, depending on the nature of the discrepancy. The first challenge is to determine whether the discrepancy involves unexpected cell or plasma reactivity. If the cells react with all antisera including an auto control and a reagent control (either albumin or a commercial control), the cells may be coated with autoantibodies, which can usually be removed by warm washing or treatment with 0.01 M dithiothreitol.² If the cells do not react with the reagent control, the problem most likely lies with the plasma. Parallel testing of the plasma with reagent screen cells may point toward a specific antibody (e.g., anti-M) or, if negative, may suggest the presence of unexpected ABO isohemagglutinins, most likely anti-A1. Testing an auto control with these screen cells is recommended, but the results must be interpreted carefully as they can be misleading. Many cold autoantibodies have IH specificity and react preferentially with type O RBCs owing to the greater expression of the H antigen on these cells.^{2,3} As a result, the auto control for a patient with blood group A, B, or AB may appear negative or much weaker, whereas all of the group O reagent cells are strongly reactive. Use of cord blood cells and donor cells of the same ABO group as the patient, and reagent panel cells negative for the I antigen can also be useful when this kind of reactivity is encountered.

Ideally, when unexpected plasma reactivity is encountered, a full panel of reagent cells should be tested at each phase of testing where reactivity is observed. When working with a limited volume of plasma, especially in the reference laboratory setting in which the patient is not readily available for a repeat blood sample collection, this ideal approach is not always possible. If reactivity is observed at multiple phases of testing, it becomes even more difficult to achieve this ideal. The use

of gel column technology requires considerably less plasma than standard tube techniques and can aid in the antibody identification process with minimal consumption of sample. Solid-phase red blood cell adherence methods, although not used in our laboratory at the time of this workup, also require less plasma than standard tube techniques and can provide similar benefits.

Closing Comments

This case served as a reminder that antibodies are not always what they initially appear to be, as when multiple alloantibodies masquerade as a cold autoantibody, and that our assumptions can potentially misdirect a workup. It also demonstrates the value of flexibility in the use of multiple tools to pursue an answer during the antibody identification process.

References

1. Immucor, Inc. Anti-H lectin package insert, revised February 2009.
2. Roback JD, Grossman BJ, Harris T, et al. Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011.
3. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. San Diego, CA: Academic Press, 2012.

Paula M.S. Wennersten, MT(ASCP)SBB CM (corresponding author), Reference Technologist, Reference and Transfusion Department, and Laurie J. Sutor, MD, MBA, Vice President of Medical and Technical Services, Carter BloodCare, 2205 Highway 121, Bedford, TX 76021; Professor, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.

***RHCE* variant allele: *RHCE**ce254G,733G**

J.A. Keller, T. Horn, C. Chiappa, C. Melland, C. Vietz, L. Castilho, and M.A. Keller

A novel *RHCE* allele was identified in a 53-year-old African-American female blood donor with an Rh phenotype of D+ C– E– c+ e+ and a negative antibody screen. The donor's cells typed e+ with all antisera tested. By gel-based genotyping and cDNA analysis, the two *RHCE* alleles in this donor were characterized. One allele was found to be the known allele *RHCE**01.20.01 (*RHCE**ce733G) and the second was novel: *RHCE**01.06.02 (*RHCE**ce254G,733G). ***Immunohematology* 2014;30:121–122.**

Case Report

A novel *RHCE* allele, *RHCE**ce254G,733G, was identified in a 53-year-old African-American female blood donor with an Rh phenotype of D+ C– E– c+ e+ and a negative antibody screen. The donor's cells typed e+ with all antisera tested. The anti-e reagents tested were Seraclone (formulated from clones MS16/MS21/MS63) (Bio-Rad Laboratories, Hercules, CA), BioClone (formulated from MS16 clone) (Ortho Clinical Diagnostics, Rochester, NY), Immucor Series 1 (formulated from MS16 clone), and Immucor Gamma-clone (formulated from clones MS16/21/63) (Norcross, GA). The hr^B status was not assessed serologically.

Materials and Methods

Genomic DNA from the donor was isolated from white blood cells using the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) and was used for genotyping of the *RHD* and *RHCE* genes using laboratory-developed gel-based genotyping assays including a multiplex *RH* polymerase chain reaction (PCR) to detect *RHD* intron 7, exon 4, *RHD* pseudogene and *RHCE* expressing the C or c antigens, restriction fragment-length polymorphism (RFLP) PCR, and sequence-specific primer (SSP) PCR. Red cell RNA isolation was performed by a laboratory-developed phenol-chloroform extraction and isopropanol precipitation method, cDNA synthesis with a Superscript III Synthesis kit (Life Technologies, Carlsbad, CA), reverse transcription–PCR amplification using *Taq* polymerase (Qiagen), and PCR product cloning with a TOPO TA cloning kit (Life Technologies). cDNA sequence analysis (cDNA seq) was performed (BigDye Terminator Kit, Life Technologies) using vector-based primers and compared with

the consensus sequence using Sequencher 5.0 (GeneCodes Corporation, Ann Arbor, MI).

Results

A variety of gel-based genotyping assays were used to screen the donor sample for known single nucleotide polymorphisms (SNPs). The results are summarized in Table 1. cDNA analysis was performed to further characterize the two *RHCE* alleles to rule out the presence of additional variants. One allele was found to be known: *RHCE**01.20.01 (*RHCE**ce733G). The second allele has not been reported previously; it carries two SNPs: *RHCE* c.254G and c.733G. The allele was named *RHCE**ce254G,733G (Table 2) based on the ISBT allele terminology.¹

Table 1. *RH* genotyping results

Gene or Gene Region	Method	Analyte	Result	Reference
<i>RHD</i> gene	Multiplex PCR	<i>RHD</i> Intron 4	present	5
		<i>RHD</i> Exon 7	present	
		Pseudogene	absent	
		C	absent	
		c	present	
	PCR	Hybrid Rhesus box	absent	6
<i>RHD</i> Exon 3	SSP	455A>C (N152T)	A	7
<i>RHD</i> Exon 5	RFLP	667T>G (F223V)	T	7
	RFLP	697G>C or A (E233Q or E233K)	G	7
<i>RHD</i> Exon 8	RFLP	1136C>T (T379M)	C	8
<i>RHCE</i> Exon 1	RFLP	48G>C (W16C)	G	9
<i>RHCE</i> Exon 2	RFLP	254C>G (A85G)	C/G	10
<i>RHCE</i> Exon 5	RFLP	676G>C (A226P)	G	11
	RFLP	733C>G (L245V)	G	12
<i>RHCE</i> Exon 7	SSP	1006G>T (G336C)	G	12
	RFLP	1025C>T (T342I)	C	13
<i>RHCE</i>	cDNA seq	cDNA	733G (plasmid type 1)	14
			254G, 733G (plasmid type 2)	

Table 2. Allele characteristics

Phenotype	Allele name	Nucleotide	Exon(s)	Amino acid(s)	Allele detail	Reference
Possible hr ^B -	<i>RHCE</i> *01.06.02	254C>G (rs57992529)	2	85 Ala>Gly	<i>RHCE</i> *ce254G,733G	2
		733C>G (rs1053361)	5	245 Leu>Val		

Additional Information

This case was the topic of a poster at the International Society of Blood Transfusion International Congress.² As per the National Center for Biotechnology Information,³ the minor allele frequency of *RHCE* c.254G and c.733G are 0.0078 and 0.0680, respectively. The frequency of the newly identified allele *RHCE**ce254G,733G is unknown, but, based on the frequency of the two SNPs, is expected to be rare. RFLP-PCR with *Hae*II can be used to interrogate *RHCE* c.254 for the presence of the variant.⁴ Although the phenotype encoded by this allele has not been fully investigated, it is noteworthy that the *RHCE**01.06 allele with only c.254G encodes a partial e antigen and an RH:-59 (CEAG-) and RH:-31 (hrB-) phenotype.

Acknowledgments

We thank the National Molecular Laboratory for performing genotyping assays.

References

1. <http://www.isbt-web.org>
2. Crowley J, Horn T, Chiappa C, et al. A novel *RHCE* allele: *RHCE**ce254G,733G. Cancun, Mexico; ISBT, 2012.
3. <http://www.ncbi.nlm.nih.gov/snp>
4. Vege S, Nickle PA, Shirey R, Westhoff CM. A novel 254C>G (Ala85Gly) change associated with partial Rhe and alloanti-e. *Transfusion* 2009;49 (Suppl.):15A.
5. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. *Blood* 2000;95:12-8.
6. Chiu RW, Murphy MF, Fidler C, Zee BC, Wainscoat JS, Lo YM. Determination of RhD zygosity: comparison of a double amplification refractory mutation system approach and a multiplex real-time quantitative PCR approach. *Clin Chem* 2001;47:667-72.
7. Reid ME, Storry JR, Sausais L, Tossas E, Rios M, Hue-Roye K, Gloster ES, Miller ST, Wolf C, Lomas-Francis C. DAK, a new low-incidence antigen in the Rh blood group system. *Transfusion* 2003;43:1394-7.
8. Westhoff CM, Vege S, Horn T, Hue-Roye K, Halter Hipsky C, Lomas-Francis C, Reid ME. *RHCE**ceMO is frequently in cis to *RHD**DAUO and encodes a hrS- hrB- RH:-61 phenotype in black persons: clinical significance. *Transfusion* 2013;53:2983-9.
9. Westhoff CM, Silberman LE, Wylie DE, Skavdahl M, Reid ME. 16Cys encoded by the *RHce* gene is associated with altered expression of the e antigen and is frequent in the R₀ haplotype. *Br J Haem* 2001;113:666-71.
10. Westhoff CM, Vege S, Halter-Hipsky C, Whorley T, Hue-Roye K, Lomas-Francis C, Reid ME. *DIIIa* and *DIII Type 5* are encoded by the same allele and are associated with altered *RHCE**ce alleles: clinical implications. *Transfusion* 2010;50S:145A.
11. Reid ME, Rios M, Powell VI, Charles-Pierre D, Malavade V. DNA from blood samples can be used to genotype patients who have recently received a transfusion. *Transfusion* 2000;40: 48-53.
12. Daniels GL, Faas BH, Green CA, Smart E, Maaskant-van Wijk PA, Avent ND, Zondervan HA, von dem Borne AE, van der Schoot CE. The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. *Transfusion* 1998;38:951-8.
13. Vege S, Meyer W, Copeland T, Westhoff CM. A new *RHce* allele, *RHCE**ceTI, is associated with C typing discrepancies and is linked to *RHD**DIVa. *Transfusion* 2007;47:159a.
14. Westhoff CM, Storry JR, Walker P, Lomas-Francis C, Reid ME. A new hybrid *RHCE* gene (*CeNR*) is responsible for expression of a novel antigen. *Transfusion* 2004;44:1047-51.

Jessica A. Keller, MS, Lead Molecular Technologist, and Trina Horn, MT(ASCP), MS, Supervisor, American Red Cross, National Molecular Laboratory; Colleen Chiappa, Bonfils Blood Center; Camilla Melland, Bonfils Blood Center, Denver, CO; Christine Vietz, Cambridge, MA; Lilian Castilho, Hemocentro-Unicamp, R. Carlos Chagas, Campinas-SP, Brazil; and Margaret A Keller, PhD (corresponding author), Director, American Red Cross, National Molecular Laboratory, 700 Spring Garden Street, Philadelphia, PA 19123.

Detection and identification of platelet-associated alloantibodies by a solid-phase modified antigen capture enzyme-linked immunosorbent assay method and its correlation to platelet refractoriness in multiplatelet concentrate–transfused patients

N. Jain, R.S. Sarkar, and J. Philip

Platelets express a variety of polymorphic glycoproteins (GPs), such as GPIIb/IIIa, GPIb/IX, GPIa/IIa, GPIV, and class I human leukocyte antigen. In the platelet transfusion setting, alloimmunization involves the production of antibodies against these glycoproteins. Patients transfused with multiple units of platelet concentrates for longer periods are the main individuals with platelet alloimmunization. This study was performed to detect the development of platelet antibodies in patients who are transfused with multiple units of leukodepleted platelet concentrates, such as those with hemato-oncologic diseases and bone marrow failure syndromes. The method used was solid-phase modified antigen capture enzyme-linked immunosorbent assay. Platelet refractoriness was assessed by measuring the corrected count increment at 1 and 24 hours after transfusion. *Immunohematology* 2014;30:123–125.

Key Words: HLA, platelet glycoproteins, platelet alloimmunization, MACE

Platelets express a variety of polymorphic glycoproteins (GPs), such as GPIIb/IIIa, GPIb/IX, GPIa/IIa, GPIV, and class I human leukocyte antigen (HLA).¹ In the platelet transfusion setting, alloimmunization involves the production of antibodies against these glycoproteins. Patients transfused with multiple units of platelet concentrates for longer periods are the main individuals with platelet alloimmunization. Approximately 20 to 85 percent of patients who receive multiple platelet transfusions become immunized against platelet antigens, and approximately 40 percent of patients who are alloimmunized exhibit refractoriness to platelet transfusions.² Among patients receiving long-term blood component support, refractoriness to platelet transfusions may be observed in 10 to 70 percent, of which HLA antibodies can be found in 20 to 70 percent of patients.³

There are three groups of assays currently available to detect platelet antibodies. The earliest were phase I assays that involved mixing patient serum with normal platelets and used platelet function–dependent end points such as alpha granule release, aggregation, or agglutination. These were followed by phase II assays that measured either surface or total platelet-associated immunoglobulin on patient or normal platelets after sensitization with patient serum. Recently, phase III assays have been developed that require the binding and detection of antibodies to isolated platelet surface glycoproteins (GPs) or HLA class-I antigens.

Materials and Methods

The study was carried out in the Department of Immunohaematology and Blood Transfusion of a tertiary-care hospital in Pune, Western India, from June 2010 to May 2012. The study was carried out after receiving approval from the institutional ethical committee and informed consent from the patients.

A total of 100 patients who had been transfused with at least 15 units of leukodepleted platelet concentrate were selected. All patients had either hematologic malignancies or bone marrow failure syndromes.

Testing was performed using the solid-phase modified antigen capture enzyme-linked immunosorbent assay (ELISA) (MACE) method, a phase III assay, in which the ELISA plate microwells are coated with platelet-specific monoclonal antibodies. The Solid Phase ELISA kit (MACE-1, Immucor GTI Diagnostics, Waukesha, WI) provides immobilized monoclonal antibodies to capture HLA class I and platelet GPIIb/IIIa. In the second kit from the same manufacturer

(MACE-2, Immucor GTI Diagnostics), the antibodies are coated onto the plate to capture GPIb/IX, GPIa/IIa, and GPIV. To detect the allogeneic platelet antibodies in the test serum, the patient sera are mixed with donor platelets and incubated for 30 minutes. Antibody, if present, will bind to the platelet glycoproteins. Unbound antibodies are washed from the platelets. The antibody-sensitized platelets are solubilized by the addition of a lysis buffer solution. The platelet lysate containing soluble glycoproteins is transferred to microwells for ELISA testing. During ELISA, platelet and HLA class-I GPs would be captured with immobilized monoclonal antibodies coated on microwells. After incubation, unbound glycoproteins are washed off. An alkaline phosphatase–labeled anti-human globulin (AHG) reagent is added and incubated. Unbound AHG is washed off. Substrate p-nitrophenyl phosphate (PNPP) is added. After 30 minutes of incubation, the reaction is stopped by using sodium hydroxide solution. The optical density of the color that develops is measured by a spectrophotometer. Confirming the presence of these antibodies in patient serum would help in the search for potentially compatible platelet components.

Results

In our study, we found that 39 of 100 (39%) of multiply transfused platelet recipients developed platelet antibodies detected by one kit or the other (Immucor GTI Diagnostics), of which 11 patients showed refractoriness. Platelet refractoriness is a clinical condition in which patients do not achieve the anticipated platelet count increment from a platelet transfusion. The Trial to Reduce Alloimmunization to Platelets study⁴ defines the refractory state as a CCI of less than 5000 after two sequential ABO-compatible platelet transfusions. Two patients did not develop platelet antibody but they showed refractoriness. The number of alloimmunized patients and the percentage manifesting refractoriness are shown in Table 1. The rate of refractoriness among our multiply transfused patient population is similar to that of other studies, which showed an incidence of refractoriness of 5 to 15 percent.^{5–7}

Table 1. Number of patients who developed refractoriness in the population of platelet alloimmunized patients

Platelet antibodies against	Number of patients who developed refractoriness/ number of patients who produced platelet antibodies
HLA-1	6/11
GPIb/IX	2/21
GPIIb/IIIa	1/2
HLA-1 + GPIb/IX	2/5

HLA = human leukocyte antigen; GP = glycoprotein.

Eleven cases of refractoriness were attributed to immunologic causes (i.e., antibody against HLA class I and HPA, human platelet antigen), and two cases were a result of some nonimmunologic causes including history of fever, sepsis, bleeding, and so forth. Of the 11 cases of immunologic etiology, two had acute myeloid leukemia, two were diagnosed with immune thrombocytopenic purpura (ITP), and two had myelodysplastic syndrome. Aplastic anemias, acute lymphoblastic leukemia, chronic myeloid leukemia, Hodgkin lymphoma, and non-Hodgkin lymphoma (NHL) were diagnosed in the remaining five cases. Three patients developed only HPA antibody, of which two had ITP and one was diagnosed with NHL.

Discussion

The risk of alloimmunization among patients receiving multiple platelet concentrate transfusions is one of the major issues of current hemotherapy. MACE is an efficient method for detection and identification of platelet antibody.

HLA sensitization is the most common immunologic cause of platelet refractoriness in multiply transfused patients and can be diagnosed by demonstration of significant levels of HLA class I antibodies in the refractory patient’s serum by MACE-1. When antibodies to HLA class I antigens are demonstrating, a widely used approach is to supply apheresis platelets from donors whose HLA class I antigens are similar to those of the patient. HLA-matched platelets should be leukodepleted to reduce the risk of alloimmunization.^{6,7} MACE-2 testing has an added advantage of selecting platelets when the antibodies involved are directed at platelet-specific, rather than HLA, antigens.

An important observation of this study is that in our patient population, the most common platelet-specific antibody detected was anti-GPIb/IX antibody. This is the first study of this kind in India.

Conclusion

In conclusion, MACE-1 and MACE-2 enabled specific detection and identification of platelet antibodies, which in turn correlated well with the development of refractoriness in multiplatelet concentrate–transfused patients. The test should specifically be used in patients who have developed platelet refractoriness to detect and identify the platelet antibodies.

References

1. Vassallo RR. Recognition and management of antibodies to human platelet antigens in platelet transfusion-refractory patients. *Immunohematology* 2009;25:119–24.
2. Slichter SJ, Kaufman RM, Assmann SF, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. *N Engl J Med* 2010;362:600–13.
3. Rebutta P. Refractoriness to platelet transfusion. *Curr Opin Hematol* 2002;9:516–20.
4. [No authors listed]. The Trial to Reduce Alloimmunization to Platelets Study Group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. *N Engl J Med* 1997;337:1861–9.
5. Murphy MF, Metcalfe P, Thomas H, et al. Use of leucocyte-poor blood components and HLA-matched-platelet donors to prevent HLA alloimmunization. *Br J Haematol* 1986;62: 529–34.
6. Laundry GJ, Bradley BA, Rees BM, Younie M, Hows JM. Incidence and specificity of HLA antibodies in multitransfused patients with acquired aplastic anemia. *Transfusion* 2004;44: 814–25.
7. Legler TJ, Fischer I, Dittmann J, et al. Frequency and causes of refractoriness in multiply transfused patients. *Ann Hematol* 1997;74:185–9.

Neelesh Jain, MD (corresponding author), Transfusion Medicine, Tata Medical Centre, Kolkata, West Bengal-700156, India; Ravi Shankar Sarkar, MD, and Joseph Philip, MD, Department of Immunohaematology and Blood Transfusion, Armed Forces Medical College, Pune, India-411040.

Notice to Readers

All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

Attention: State Blood Bank Meeting Organizers

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please send request, 4 months in advance, to immuno@redcross.org

For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, **contact** Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at Sandra.Nance@redcross.org

Immunohematology is on the Web!

www.redcross.org/about-us/publications/immunohematology

For more information, send an e-mail to immuno@redcross.org

Effects of pH changes of stock normal saline solution on 5 percent red cell suspension

G.L. Martin, P.J.M. Caraan, J.J.S. Chua, J.A.L. Crescini, J.M.C. Diokno, C.B.Dlr. Javier, M.K.B.O. Reyes, and R.Y. Soliven

The red cell suspension (RCS) is a universally used indicator system to demonstrate antigen and antibody reactions in vitro. Saline solutions that are used in its preparation are preferred to be fresh to avoid changes in pH that may affect the results. Thus, buffered saline such as phosphate-buffered saline (PBS) is the ideal diluent because its pH is maintained for a certain period. However, normal saline solution (NSS) is more commonly used because it is inexpensive and easy to make. pH changes in the saline solutions and the RCSs were monitored for 1 week. Macroscopic examination of changes in degree of redness of RCS was also observed. Red blood cell (RBC) indices of the ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood used in the preparation of the RCS were measured in the performance of an automated complete blood count. Qualitative examination of the crenation of RBCs was done on the prepared blood smears and graded by three registered medical technologists. Percentage crenation was then determined using an improved Neubauer counting chamber. Three trials were performed, and results were averaged. Statistical analysis showed that there were significant differences in the average pH of PBS and NSS and the average pH of RCS-PBS and RCS-NSS over 1 week. RBC indices measured in EDTA-anticoagulated donor blood showed no significant difference. Macroscopic examination of changes in degree of redness of the RCS showed that color darkened over 1 week but only by a small degree. Qualitative and quantitative examination of crenation of RBCs in RCS-PBS and RCS-NSS both showed no significant differences over 1 week. The experimental group (RCS-NSS) continuously showed a higher grade of crenation than the control group (RCS-PBS). Crenation of RBCs still manifests microscopically despite the lack of a significant relationship between the pH of the saline solutions and the degree and percentage of crenation. Crenation, therefore, cannot be attributed to pH alone but occurs as a result of other factors. *Immunohematology* 2014;30:126–134.

Key Words: phosphate-buffered saline, normal saline solution, red cell suspension, red blood cell indices, pH changes

The red cell suspension (RCS) is a universally used indicator system to demonstrate antigen and antibody reactions in vitro. Saline solutions that are used in its preparation are preferred to be fresh to avoid changes in pH that may affect the results. Thus, buffered saline such as phosphate-buffered saline (PBS) is the ideal diluent because its pH is maintained for a certain period. However, normal saline solution (NSS) is more commonly used because it is inexpensive and easy to make.

One of the reagents that should be readily available in a laboratory is saline. Saline refers to the solution of sodium

chloride (NaCl; common table salt) in water that exists in various concentrations and formulations and is very essential in the performance of laboratory procedures. Saline is the ideal diluent used in blood bank procedures because it is isotonic in nature and will not cause hemolysis of red blood cells (RBCs). Intact erythrocytes maintain normal shape in the cellular pH range of 6.3 to 7.9, but invaginate at lower pH values. Below an external pH of 6.0, membrane curvature becomes negative (stomatocytic); above pH 8.0, curvature becomes positive (echinocytic). It was shown that cytoplasmic pH is the primary physiological mediator of RBC shape change in altered pH buffers.²

Isotonic saline is used to wash RBCs when preparing an RCS. This RCS is used as a source of antigen to which the corresponding antibody can react to produce agglutination or hemolysis in vitro. This suspension exists in different concentrations (2%, 3%, 4%, 5%) and is prepared by using anticoagulated blood.³ A 2 percent to 5 percent RCS provides optimal antigen concentration for various blood bank laboratory applications such as ABO typing and crossmatching. RBCs are washed with saline to remove traces of plasma that may contain substances that may interfere with antigen–antibody reaction. These substances include soluble antigens such as A and B, which when present may neutralize the reagent; interfering proteins such as Wharton's jelly, which may cause red cells to nonspecifically aggregate; and other rouleaux-promoting substances such as globulins and fibrinogen, which when elevated make interpretation of reactions difficult and misleading.³

Saline solutions are preferably freshly prepared because washing RBCs with stock saline may yield a pH below or above the normal range required and may cause inaccurate results.⁴ Two common formulations of saline solutions are NSS and PBS. NSS (physiologic saline or isotonic solution) is chemically 0.85 percent or 0.90 percent w/v NaCl, which is about 300 mOsm/L, an osmolarity similar to the osmolarity of this salt in blood. PBS solution, on the other hand, is a water-based salt solution containing sodium chloride, sodium phosphate, and, in some formulations, potassium chloride and potassium phosphate. The buffer's phosphate groups help maintain pH

between 7.2 and 7.4. The osmolarity and ion concentration of the solution usually match those of the human body (isotonic).

PBS is the ideal diluent because its pH is maintained for a certain time period. However, schools and laboratories, especially those from government hospitals, commonly use NSS because it is inexpensive and easy to make. The use of PBS for daily procedures may be impractical because of its cost. Thus, it is preferable to purchase a cheaper, ready-made 0.85 percent to 0.9 percent NSS or make one by dissolving 0.85 to 0.90 g of NaCl in enough volume of distilled water to reach 100 mL. The NSS—regardless of whether it is purchased from pharmaceutical companies or traditionally made, kept, and stored until consumed—may have pH variations that cannot be controlled because it is unbuffered. In this context, these changes in pH may have effects on RBCs that are yet to be explored in this study.

Materials and Methods

Research Locale

The research experiment was conducted at the San Lazaro Hospital Laboratory, Philippines. The laboratory provided most of the necessary equipment and ancillary instruments needed by the researchers such as the automated Complete Blood Count (CBC) analyzer, laboratory centrifuge, microscope, and refrigerator. All other materials such as test tubes, beakers, amber bottles for storage of saline solution, hemocytometer, glass slides and cover slips, and others were provided by the researchers.

Research Design

The study was of the experimental type and involved the collection of donor samples, preparation of saline solutions and subsequent preparation of RBC suspensions, and measurement and examination of defined RBC parameters (Fig. 1.) resulting in a comparative observation and analysis between two commonly used saline solutions—PBS and NSS. PBS served as the control and NSS as the test sample. One liter of PBS was purchased from University of Santo Tomas Laboratory Equipment and Supplies Office (UST-LESO), and 1 L of NSS was also prepared by dissolving 8.50 to 9.0 g of chemical-grade NaCl in enough distilled water to reach 1000 mL.

The pH of both saline solutions was recorded at 0, 2, 4, 6, 8, 24, 48, 72, 96, 120, and 144 hours (Table 1). The procedure was performed for 1 week.

Two 5 percent RCSs washed three times with PBS and NSS were made from ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood from a specific donor. RBC

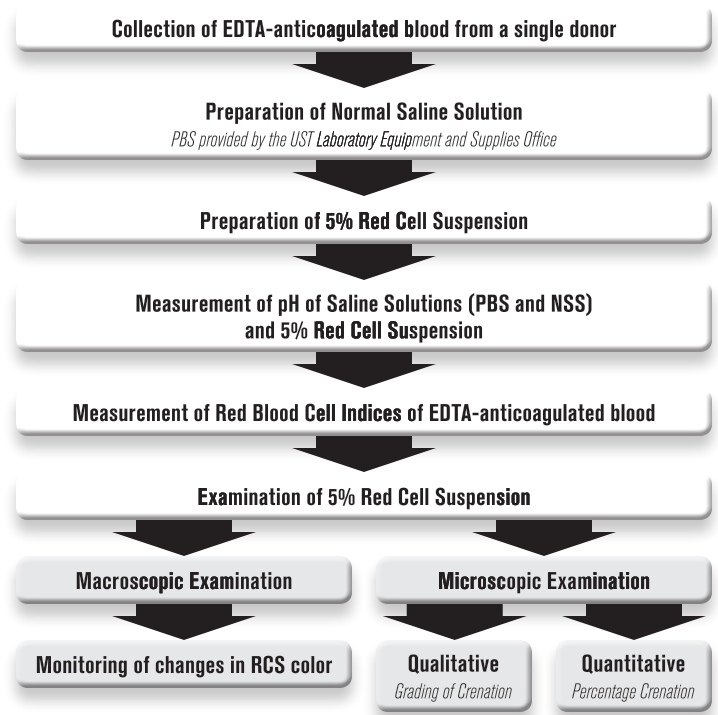


Fig. 1 Flowchart of the research procedure. EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline; NSS = normal saline solution; RCS = red cell suspension.

Table 1. Time schedule of the research procedure

0 h	2 h	4 h	6 h	8 h	24 h	48 h	72 h	96 h	120 h	144 h
Day 1					Day 2	Day 3	Day 4	Day 5	Day 6	Day 7

indices of this collected blood were also measured, monitored, and documented to determine cellular changes undergone by the erythrocytes.

The intensity of redness of RCSs was observed using a comparator chart (Fig. 2). Moreover, morphologic changes of RBCs were also determined by grading the degree of crenation with time (Fig. 3). This was then evaluated by three registered medical technologists, and the percentage of crenated RBCs was computed. Three trials were performed, and results were averaged.

COLLECTION OF DONOR-SPECIFIC EDTA-ANTICOAGULATED BLOOD

Venous blood was collected from the antecubital vein and transferred to an EDTA-anticoagulated tube. The blood collected from a specific donor was used from the beginning to the end of the study. The donor specificity minimized unnecessary variables and controlled external factors that could yield inaccurate results. The donor sample was subjected to a CBC beforehand to ensure that the study was performed

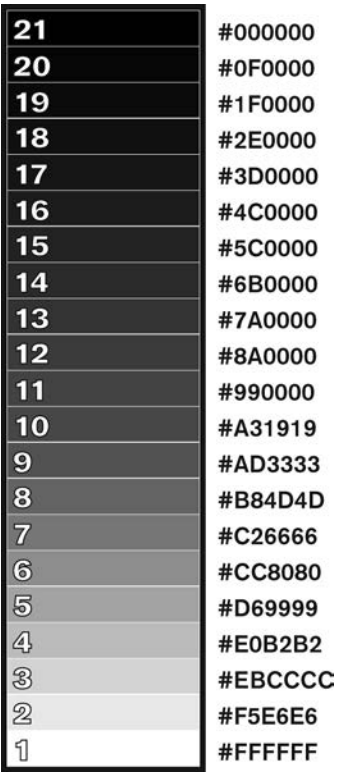


Fig. 2 A comparator chart showing degrees of redness 2012 Refsnes Data.

under normal conditions with time.

PREPARATION OF 0.85 PERCENT TO 0.9 PERCENT NSS

NSS served as the diluent to be tested and was prepared by weighing 0.85 to 0.90 g of chemical-grade NaCl or pure table salt and dissolving it in distilled water. The researchers prepared 1 L of NSS; hence, 8.50 to 9.00 g of chemical-grade NaCl was dissolved in enough distilled water to reach the 1000-mL mark in the volumetric flask. The prepared NSS was stored at refrigerator temperature (between 2°C and 6°C) and used from the beginning to the end of the study.

PREPARATION OF 5 PERCENT RCS

The donor-specific EDTA-anticoagulated blood was apportioned into two aliquots, transferred into two clean test tubes, and labeled accordingly: “Blood for RCS-PBS” (blood to be used for RCS washed with PBS) and “Blood for RCS-NSS” (blood to be used for RCS washed with NSS). Blood for RCS-PBS and blood for RCS-NSS were washed with PBS and NSS, respectively, and centrifuged for 1 minute at 3400 rpm. The supernatant was decanted by quick inversion. The process was performed three times. After the last washing, the supernatant was removed completely by using a Pasteur pipette so as to not disturb the cell button.

RCS exists in different concentrations (2%–5%) and in different volumes; however, the study focused on the preparation of a 5 percent RCS. Twenty milliliters of 5 percent RCS was prepared by the researchers. To determine the volume of washed packed RBCs (WPRBC) used, the total volume of RCS to be prepared (20 mL) was multiplied by the desired concentration (5%) and divided by 100. Thus, 1.0 mL of WPRBC was used by the researchers in the study. Using an automatic pipette with 1.0-mL capacity, 1.0 mL of distilled water was aspirated and delivered into a clean identical test tube with same volume capacity. The 1.0-mL level was marked, and the test tube was set aside to serve as a tared tube for the 1.0 mL of WPRBC. Using a clean Pasteur pipette, WPRBC were aspirated and dropped into a clean separate test tube until they reached the volume equal to the 1.0-mL level of the tared test tube.

To determine the volume of NSS or PBS added to 1.0 mL of WPRBC, the total volume of WPRBC (1.0 mL) was subtracted from the total volume of the RCS to be prepared (20 mL). Nineteen milliliters of NSS or PBS was aspirated using a pipette and added to 1.0 mL of WPRBC. A proper RCS exhibited a tomato-red color.

The prepared RCSs and saline solutions (PBS and NSS) were stored in the blood bank refrigerator at 2° to 6°C immediately after use because temperature instability may greatly affect RBC membrane deformability and, therefore, the stability of the membrane during processing.⁵

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF 5 PERCENT RCS WASHED WITH PBS (CONTROL) AND NSS (EXPERIMENTAL) AS DILUENTS

The pH of the saline solutions and their corresponding RCS preparations and RBC indices (mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration) of the EDTA-anticoagulated blood used in the preparation of the RCS were measured for 1 week. Examination of the 5

GRADING	MICROSCOPIC APPEARANCE	DESCRIPTION
0		No visible crenations of red blood cells
±		About 20% of the red blood cells are crenated
+1		About 40% of the red blood cells are crenated
+2		About 60% of the red blood cells are crenated
+3		About 80% of the red blood cells are crenated
+4		100% of the red blood cells are crenated

Fig. 3 Grading scale for degree of red blood cell crenation.

percent RCS was divided into macroscopic and microscopic examination. The macroscopic examination involved observation of RCS change in degree of redness with time. The microscopic examination involved determining the presence or absence of RBC crenation and subsequent grading of degree of crenation by three registered medical technologists and computing the percentage of crenated cells.

The parameters were measured for 1 week with a 2-hour interval for the first 8 hours. The pH of the saline solutions (tubes labeled PBS and NSS) and an aliquot portion of the prepared 5 percent RCSs (tubes labeled RCS-PBS and RCS-NSS) were measured before measurement of other parameters using a precalibrated pH meter with an accuracy of ± 0.02 . The change in the degree of redness of the RCS (tubes labeled RCS-PBS and RCS-NSS) was measured using a comparator chart showing increasing and decreasing degrees of redness. RBC indices of the EDTA-anticoagulated blood were measured by performing a CBC to ensure that the researchers were working on normal RBCs based on size and amount of hemoglobin present.

The presence and absence of crenation was microscopically examined and subsequent grading of degrees of crenation followed. This step was done by preparing two RCS smears (PBS-RCS smear and NSS-RCS smear). One drop each of RCS-PBS and RCS-NSS was placed on two separate clean glass slides and secured with a coverslip. RBC morphology was examined under a high-power objective. Crenation of RBCs was graded by three registered medical technologists using an arbitrary scale (Fig. 3). This RBC crenation scale was determined based on a 7-day pre-experiment of an NSS-RCS smear observing the changes in RBC morphology under a high-power objective over time.

The change in degree of redness of the RCS over a weeklong interval was measured by this comparator chart showing different degrees of redness ranging from black (#000000) to a standard tomato-red color (#990000) to white (FFFFFF) (Fig. 4). At day 1, 0 hours, both RCS-PBS and RCS-NSS exhibited a standard tomato-red color. Only one researcher was tasked to observe the changes in degree of redness of the RCS to minimize interobserver variability.

Quantitative examination of the crenated RBCs involved counting the number of crenated cells in five fields in the central secondary square of the improved Neubauer (labeled R1–R5). The total number of crenated RBCs was divided by the total number of RBCs counted in the same fields and multiplied by 100 to obtain the percentage. The RCS was diluted with the respective saline solution at a ratio of 1:4. A dilution was performed to allow the researchers to examine

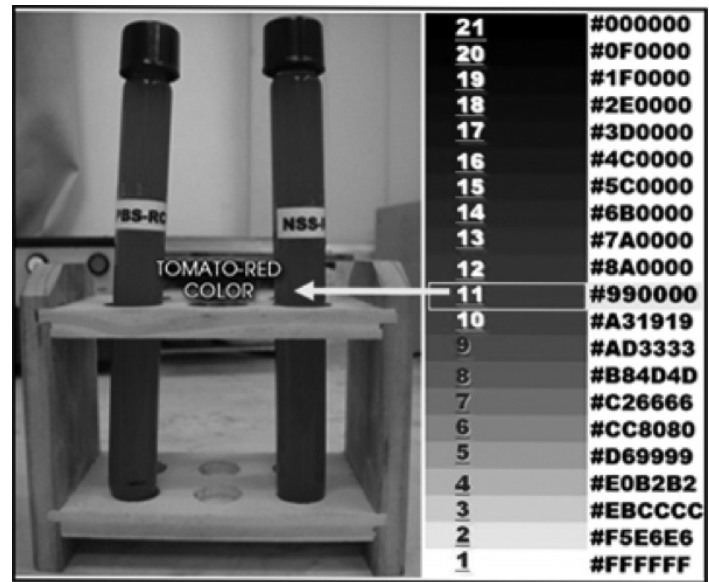


Fig. 4 Comparator chart showing degrees of redness 2012 Refsnesc Data. Reference number 11 was established after preparing the 5 percent RCSs using both PBS and NSS as diluents and comparing their results with those of the comparator chart. RCS = red cell suspension; PBS = phosphate-buffered saline; NSS = normal saline solution.

the morphology of RBCs more clearly. Counting chambers were allowed to stand for 2 minutes. Counting of crenated RBCs was not performed immediately because it was observed that crenation tends to increase with prolonged time after the setup of the counting chamber. Hence, high-quality pictures of the five fields were taken and counting was done on the photos instead. Only one researcher was tasked to count and compute the percentage of crenation to minimize interobserver variability.

Statistical Analyses

Numerical and ordinal data were used in the statistical analyses of the study. Numerical data such as pH of the saline solutions, their corresponding RCS preparations, and quantitative examination (percentage crenation) used parametric measurements involving Pearson correlation coefficient to determine the relationship between the control group (PBS) and the experimental group (NSS), and an independent Student's *t* test was used to determine significant differences between the two variables.

Nominal data such as the changes in degree of redness of RCS and qualitative examination (grading of crenation) used nonparametric measurements involving Mann-Whitney *U* test to test for significant differences between the control and experimental groups.

Statistical software (SPSS 17.0, SPSS, Inc., IBM Corp., Armonk, NY) was used to perform the statistical tests. Probability values of less than 0.05 indicate significant difference and association.

Results and Discussion

pH Changes of Saline Solutions and Their Corresponding RCS Preparations

The pH of the saline solutions (PBS and NSS) and their corresponding RCSs (RCS-PBS and RCS-NSS) were measured using a calibrated pH meter with an accuracy of ± 0.02 . Figure 5 shows the average pH of PBS and NSS over the course of 1 week. It shows that PBS exhibits a higher pH than NSS with the exception of the 2nd and 48th hours. Moreover, RCS-PBS exhibits a higher pH than RCS-NSS at all times, as seen in Figure 6.

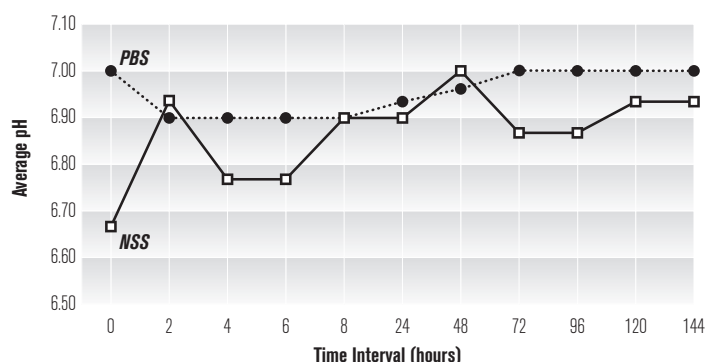


Fig. 5 Graphical representation of average pH of PBS (control) and NSS (experimental) during 1 week. PBS = phosphate-buffered saline; NSS = normal saline solution.

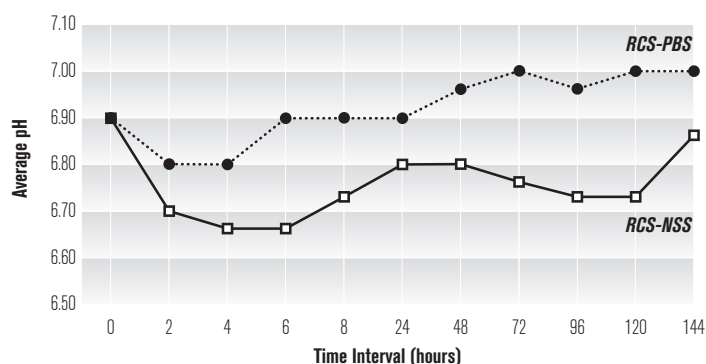


Fig. 6 Graphical representation of average pH of RCS washed with PBS (control) vs. RCS washed with NSS (experimental) during 1 week. RCS = red cell suspension; PBS = phosphate-buffered saline; NSS = normal saline solution.

There are significant differences between the control group and the experimental group in 1 week with computed

probability values of less than 0.05; however, the PBS control and NSS experimental solutions maintained their effectiveness as diluents in terms of pH because their values are still within their normal pH ranges at the end of 1 week (PBS = 7.0–7.4; NSS = 4.5–7.0).

pH of PBS and NSS in Relation to pH of RCS-PBS and RCS-NSS

Statistical analysis was done by Pearson correlation coefficient, and a computed probability value of 0.002 indicates a significant relationship between PBS and RCS-PBS. As the pH of PBS increases, the pH of its RBC suspension preparation (RCS-PBS) likewise increases.

Figure 7 shows that over time PBS exhibits a higher pH with a likewise higher pH of RCS-PBS; hence, the upward linear line denotes a direct relationship between the two variables. A computed correlation coefficient of 0.812 between PBS and RCS-PBS shows a high degree of relationship between the two variables.

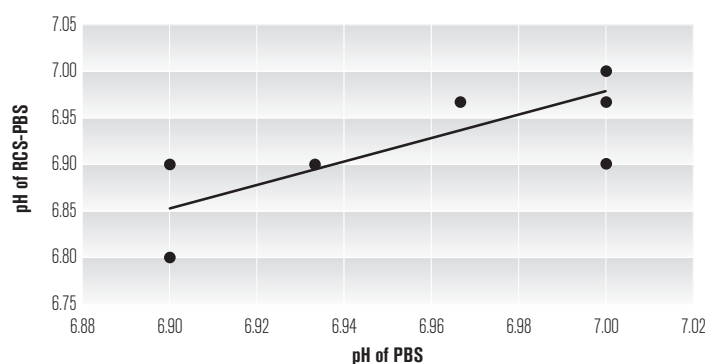


Fig. 7 Graphical representation of the pH of PBS in relation to pH of RCS-PBS during 1 week. PBS = phosphate-buffered saline; RCS = red cell suspension.

For the experimental group (NSS), a computed probability value of 0.894 indicates no significant relationship between NSS and RCS-NSS. A low degree of relationship between the pH of NSS and the pH of RCS-NSS is shown by the correlation coefficient of -0.046 . Over time, a higher pH of NSS is usually associated with lower pH values of RCS-NSS, hence the inverse relationship (Fig. 8).

RBC Indices

RBC indices were measured by performing a CBC on the EDTA-anticoagulated blood used in the preparation of the RCS. The indices were measured to ensure that the researchers were working on blood with normal RBCs based on size and amount of hemoglobin.

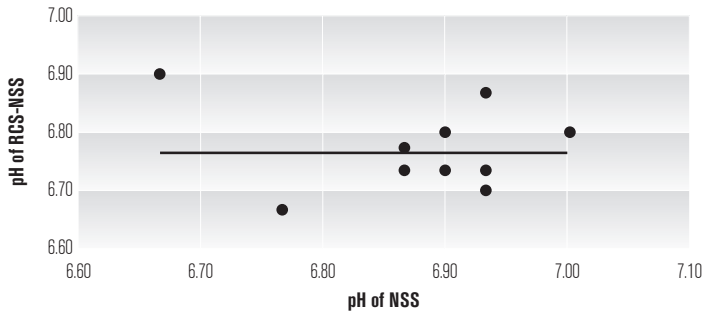


Fig. 8 Graphical representation of the pH of NSS in relation to pH of RCS-NSS during 1 week. NSS = normal saline solution; RCS = red cell suspension.

Statistical analysis through Pearson correlation coefficient showed no significant association between the RBC indices (mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration) and the pH of the saline solutions (PBS and NSS) with a computed probability value of greater than 0.05 (Fig. 9, Tables 2–4).

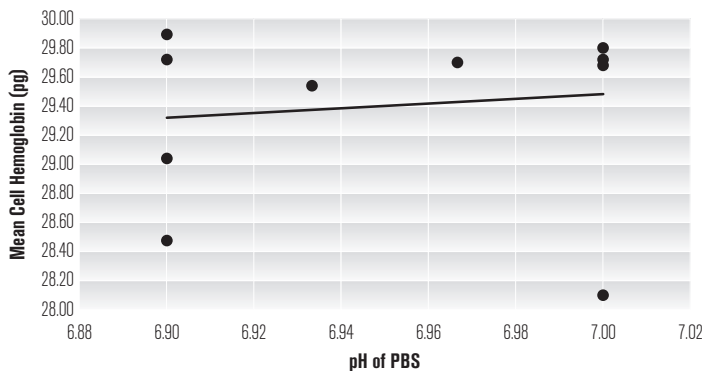


Fig. 9A Scatter plot showing the relationship between the mean cell hemoglobin of the EDTA-anticoagulated blood used and the pH of PBS during 1 week. PBS = phosphate-buffered saline.

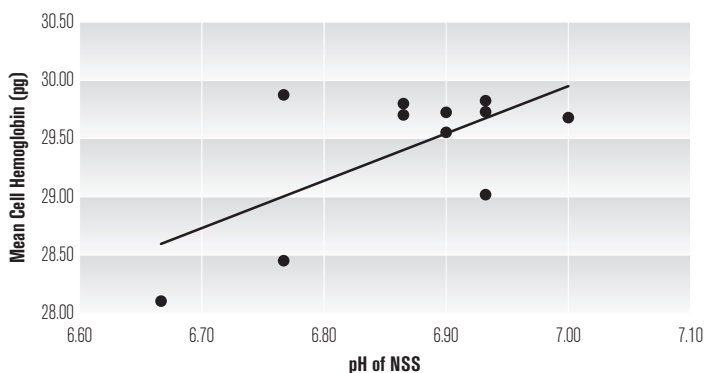


Fig. 9B Scatter plot showing the relationship between the mean cell hemoglobin of the EDTA-anticoagulated blood used and the pH of NSS during 1 week. EDTA = ethylenediaminetetraacetic acid; NSS = normal saline solution.

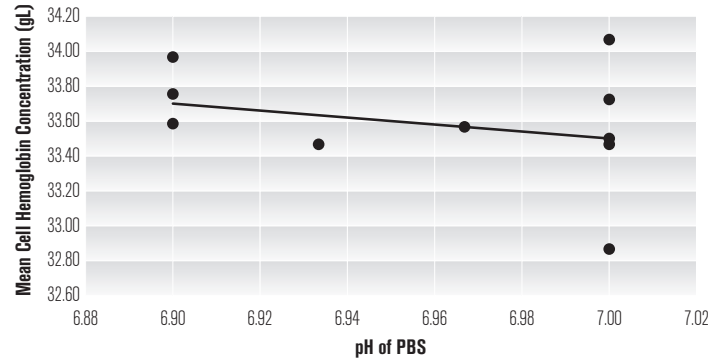


Fig. 9C Scatter plot showing the relationship between the mean cell hemoglobin concentration of the EDTA-anticoagulated blood used and the pH of PBS during 1 week. EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline.

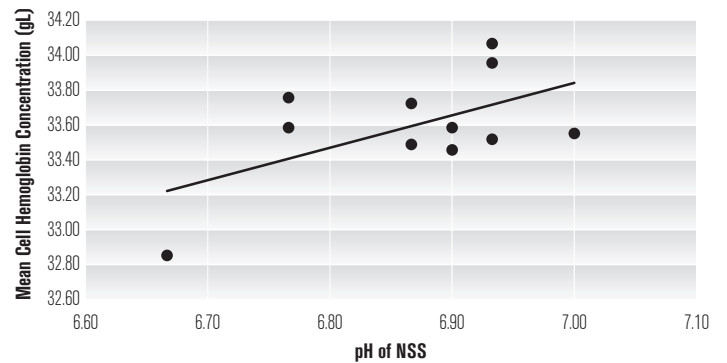


Fig. 9D Scatter plot showing the relationship between the mean cell hemoglobin concentration of the EDTA-anticoagulated blood used and the pH of NSS during 1 week. EDTA = ethylenediaminetetraacetic acid; NSS = normal saline solution.

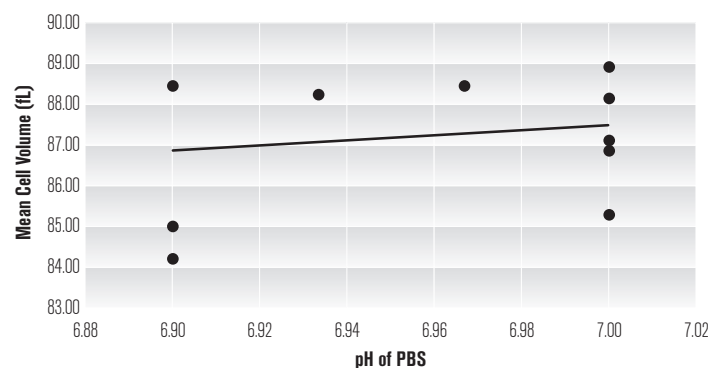


Fig. 9E Scatter plot showing the relationship between the mean cell volume of the EDTA-anticoagulated blood used and the pH of PBS during 1 week. EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline.

Changes in Degree of Redness of RCS

Figure 10 shows the changes in degree of redness of the RCS preparations, which was determined by means of a comparator chart (refer to Fig. 4) with 11 (#990000) as the

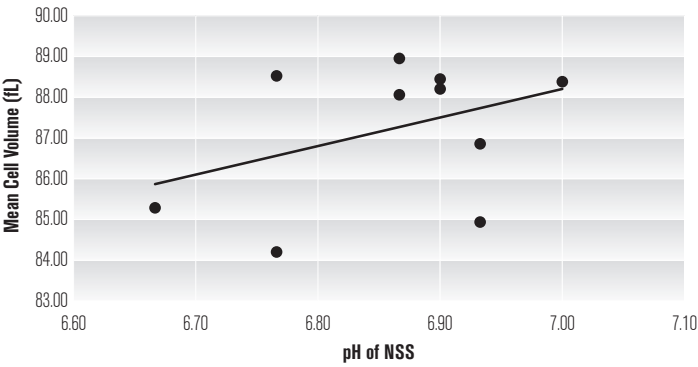


Fig. 9F Scatter plot showing the relationship between the mean cell volume of the EDTA-anticoagulated blood used and the pH of NSS during 1 week. EDTA = ethylenediaminetetraacetic acid; NSS = normal saline solution.

Table 2. Mean cell hemoglobin of EDTA-anticoagulated blood from a single donor in relation to pH of the saline solutions (PBS and NSS)

Mean cell hemoglobin	Correlation coefficient	p value	Verbal interpretation
pH of PBS	0.171	0.615	Not significant
pH of NSS	0.148	0.664	Not significant

EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline; NSS = normal saline solution.

Table 3. Mean cell hemoglobin concentration of EDTA-anticoagulated blood from a single donor in relation to pH of the saline solutions (PBS and NSS)

Mean cell hemoglobin concentration	Correlation coefficient	p value	Verbal interpretation
pH of PBS	-0.024	0.332	Not significant
pH of NSS	0.064	0.433	Not significant

EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline; NSS = normal saline solution.

Table 4. Mean cell volume of EDTA-anticoagulated blood from a single donor in relation to pH of the saline solutions (PBS and NSS)

Mean cell volume	Correlation coefficient	p value	Verbal interpretation
pH of PBS	0.029	0.932	Not significant
pH of NSS	0.018	0.957	Not significant

EDTA = ethylenediaminetetraacetic acid; PBS = phosphate-buffered saline; NSS = normal saline solution.

starting point (tomato-red color). Both RCS-PBS and RCS-NSS darkened by two degrees (from 11 to 13) in 1 week. The standard tomato-red color was maintained during the first 24 hours in both RCS preparations.

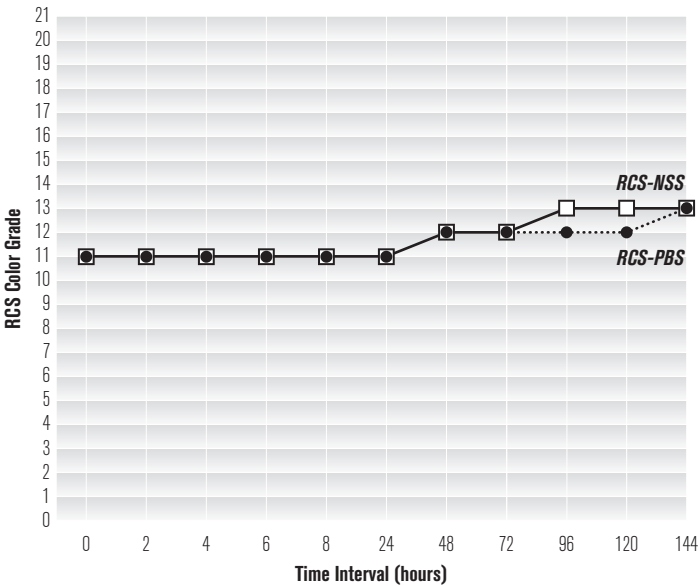


Fig. 10 Line graph showing changes in degree of redness of RCS-PBS and RCS-NSS during 1 week. RCS = red cell suspension; PBS = phosphate-buffered saline; NSS = normal saline solution.

In general, it shows that RBCs undergo lysis during 1 week regardless of the control of external factors as evidenced by the change in the degree of redness from the comparator chart.

Qualitative Examination of Grading of Crenation of RBCs

Figure 11 shows that RCS-NSS exhibits a higher degree of RBC crenation than RCS-PBS from 0° through 120°.

Statistical analysis of the relationship between the pH of the saline solutions and the grading of RBC crenation was done by computation of mean rank and Mann-Whitney *U* test. Both groups, control and experimental, show no significant relationship with a computed probability value of greater than 0.05 (Table 5). Nevertheless, the pH of the saline solutions used

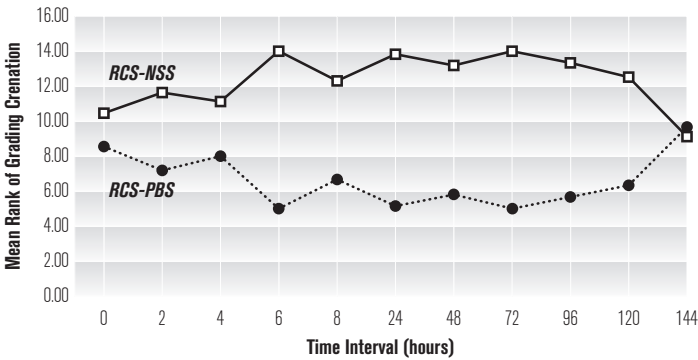


Fig. 11 Graphical representation of the degree of crenation of RCS-PBS and RCS-NSS during 1 week. RCS = red cell suspension; PBS = phosphate-buffered saline; NSS = normal saline solution.

Table 5. pH of the saline solutions used (PBS and NSS) in relation to the degree of crenation of its corresponding red cell suspensions (RCS-PBS and RCS-NSS)

Parameters	Correlation coefficient	<i>p</i> value	Verbal interpretation
pH of PBS vs. pH of RCS-PBS	0.179	0.136	Not significant
pH of NSS vs. pH of RCS-NSS	0.048	0.213	Not significant

PBS = phosphate-buffered saline; NSS = normal saline solution; RCS = red cell suspension.

and the degree of crenation of their corresponding RCSs shows a low degree of relationship at the 0.05 α level of significance (Table 3). As the pH of both saline solutions used increases, the degree of crenation of their corresponding RCSs also increases.

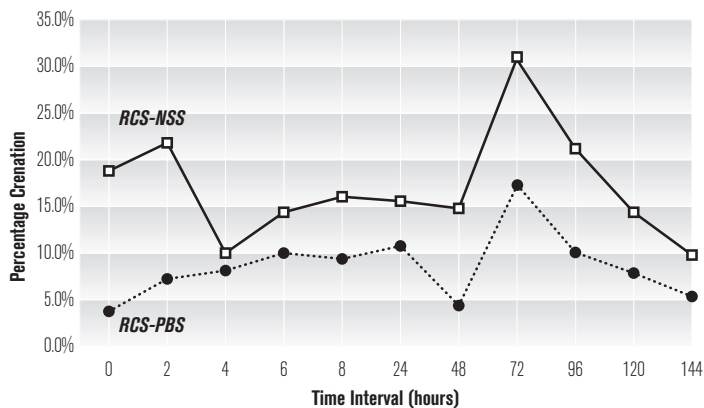


Fig. 12 Graphical representation of the average percent crenation of RCS-PBS and RCS-NSS during 1 week. RCS = red cell suspension; PBS = phosphate-buffered saline; NSS = normal saline solution.

Quantitative Examination of Percentage Crenation of RBCs

Figure 12 shows that in terms of the average percent crenation, the experimental group (RCS-NSS) continuously showed a higher percentage than the control group (RCS-PBS).

The relationships between the percent crenation of RBCs and the pH of the saline solutions used were also statistically analyzed. With a probability value of 0.433 for the control group and a probability value of 0.917 for the experimental group, the null hypothesis is accepted; that is, there is no significant relationship between the pH of PBS and the percent crenation of RCS-PBS and also between the pH of NSS and the percent crenation of RCS-NSS. However, the lack of a significant relationship does not suggest that there is no relationship that exists between the parameters being compared. Both PBS and NSS show a low degree of relationship with the percent crenation of their corresponding RCSs. Scatter plots are presented in Figure 13 to show the relationships that exist between the

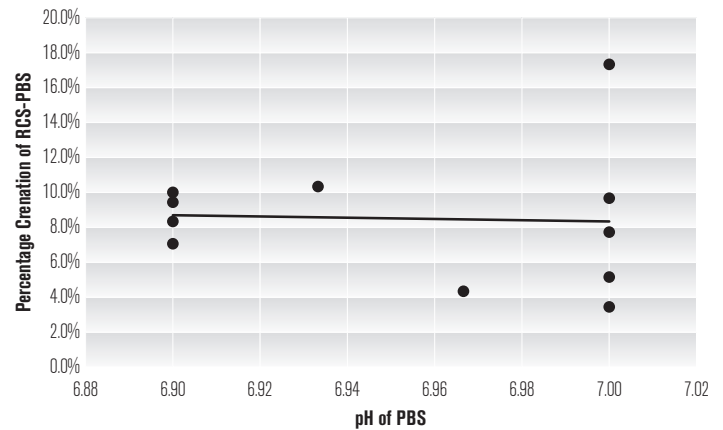


Fig. 13A Graphical representation of the pH of PBS in relation to the percent crenation of RCS-PBS. PBS = phosphate-buffered saline; RCS = red cell suspension.

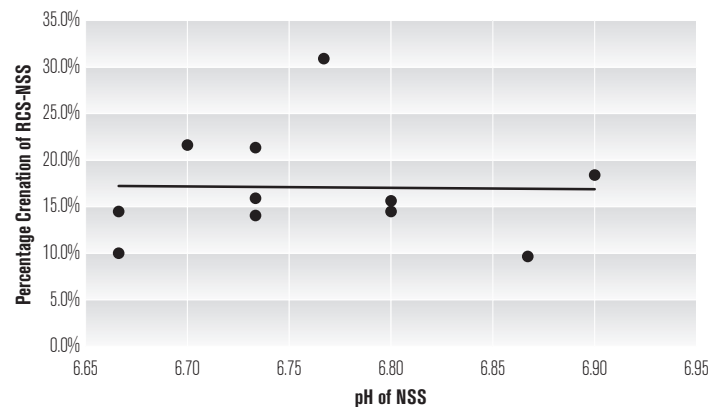


Fig. 13B Graphical representation of the pH of NSS in relation to the percent crenation of RCS-NSS. NSS = normal saline solution; RCS = red cell suspension.

pH of the diluent and the percent crenation observed in the two RCSs prepared. Figure 13A shows a negative relationship between the pH of PBS and the percent crenation of RCS-PBS ($r = -0.264$), indicating that an inverse relationship exists between the two. That is, when the pH of PBS increases, the percent crenation of RCS-PBS decreases. Furthermore, Figure 13B also shows a negative relationship between the pH of NSS and the percent crenation of RCS-NSS ($r = -0.036$), signifying that there is an inverse relationship between the two variables. It is hypothesized therefore that even if the pH is high but still falls within the range of the ideal pH of buffered saline (7.2–7.4), crenation is less likely to happen. However, this has to be further investigated considering that other factors may have contributed to such phenomena of inhibition of crenation.

Conclusions

Based on the results obtained, there was a significant difference between the pH of PBS and NSS and the pH of RCS-PBS and RCS-NSS during 1 week. Macroscopic examination of changes in degree of redness showed that the tomato-red color darkened with time (two-degree increase), thus suggesting hemolysis of RBCs is occurring. RBC indices did not change significantly. Qualitative examination of crenation showed that RBCs in RCS-NSS showed a higher grading of crenation than those in RCS-PBS. The percent crenation of RBCs in RCS-NSS showed no significant change in 1 week. There was no distinct trend in the pH of saline and its RCS preparation. Likewise, crenation of RBCs showed a rise and fall trend with time. Crenation of RBCs in the RCS preparations (RCS-PBS and RCS-NSS) therefore cannot be attributed to pH alone but to several factors other than pH.⁶ These may include factors such as incubation at various temperatures, high levels of fatty acids, the senescent state of RBCs, saline washings, or, lastly, the glass effect.

Crenation of RBCs in the experiment may be attributed to the use of glass slides and cover slips in the qualitative examination and the use of the improved Neubauer counting chamber and a thick cover slip for the quantitative examination. It was observed that crenation did not manifest immediately but only after time.

Recommendation

Although the current research has shown that pH change did not have any statistically significant effect on degree of crenation of RBCs in the 5 percent cell suspensions, the following are still recommended for future research: (1) application of RBCs washed with stock NSS for different laboratory procedures like ABO blood grouping and crossmatching, (2) determination of different stages of RBC crenation with time as observed in scanning electron microscopy to highlight detailed morphologic changes occurring, (3) effects of other external and internal factors on crenation (i.e., different lengths of time for standing of blood smears [for qualitative and quantitative evaluation of percent crenation], incubation time, temperature of experimental site, storage time of unbuffered saline solutions, pH stability of diluents for washing and cell suspensions, high ratio of glass to RBCs and close contact of RBCs to glass slide and cover slip on RBCs, type of anticoagulated blood used, degree of dissolution of salt in the diluent to explain variability in pH of the two different diluents), (4) measurement of RBC indices on the

RCSs, (5) effects of the degree of crenation with the expressed antigens on RBCs and its effect on the degree of antigenicity, and, lastly, (6) effects of using two different diluents on RBC antigens of other major blood group systems such as Rh, Kell, MNSs, Duffy, and Kidd.

Acknowledgments

We express our sincere gratitude to Ms. Angelica Fabillar and Mr. Xandro Alexi A. Nieto for providing statistical analyses of our data. We thank San Lazaro Hospital Laboratory for allowing us to conduct our experimentation in their blood bank laboratory. We also thank Mr. Mark Lester Lopez, RMT, Ms. Mary Jhoydee Julao, RMT, and Mr. Aron Patnon, RMT, for their assistance in the qualitative examination of crenation of RBCs, which is an inestimable contribution to the completion of this paper.

References

1. Cardona C, Martin G, Garcia R. Laboratory manual in blood banking. Quezon City: C & E Publishing House, 2011:1–9.
2. Gedde MM, Yang E, Huestis WH. Resolution of the paradox of red cell shape changes in low and high pH. *Biochim Biophys Acta* 1999;1417:246–53.
3. Harmening D. Modern blood banking and transfusion services. 5th ed. Philadelphia, PA: F.A. Davis Company, 2005.
4. Montés i Micó R, López-Alemán A. Comparative study of the pH over time of use of contact lens saline solutions. *Int Contact Lens Clin* 1998;25:11–15.
5. Sowemimo-Coker SO. Red blood cell hemolysis during processing. *Transfus Med Rev* 2002;16:46–60.
6. Brecher G, Bessis M. Present status of spiculed red cells and their relationship to the discocyte-echinocyte transformation: a critical review. *Blood* 1972;40:333–44.

Gregorio L. Martin, RMT, MSMT, MPH (corresponding author), Faculty Member/Blood Bank Course Lecturer, 118-D General Luna Street, Caloocan City, Philippines; Princess Joy M. Caraan, BSMT, Batangas Philippines; Jared Jenrik S. Chua, BSMT, Manila, Philippines; Jules Albert L. Crescini, BSMT, Manila, Philippines; Joseph Martin C. Diokno, BSMT, Manila, Philippines; Christopher Bernard Dlr. Javier, BSMT, Manila, Philippines; Kristina Barbara O. Reyes, BSMT, Manila, Philippines; Rianne Y. Soliven, BSMT, University of Santo Tomas Faculty of Pharmacy, Department of Medical Technology, Manila, Philippines.

Unusual erythrocyte split chimerism in pregnancy after allogeneic stem cell transplantation

Increasing numbers of successful pregnancies have been reported in recipients of allogeneic hematopoietic stem cell transplantation (HSCT). Unique to the HSCT scenario, the host blood and immune system is replaced by that of an allogeneic donor, and there are not much data available to help fully understand the mechanism involved when pregnancy and transplantation are correlated.¹ Herein we report a case of a pregnant woman after an allogeneic HSCT for aplastic anemia, with an unusual and transient split erythrocyte chimerism detected during routine ABO blood grouping for prenatal tests.

A 25-year-old woman with a history of severe aplastic anemia after a miscarriage received a myeloablative conditioning regimen (cyclophosphamide, 200 mg/kg; busulfan, 4 mg/kg) followed by the infusion of unmanipulated bone marrow cells from an HLA-identical sibling. The patient was blood group B and the donor was group O (minor incompatibility); however, both had the R₁R₂;K-k+;M+N+S-s+;Fy(a-b-) phenotype. After HSCT, cyclosporine was maintained for 2 years to prevent rejection. The patient had been seen by a hematologist monthly for 3 years and thereafter every 6 months; routine examinations were required including ABO grouping (gel and tube tests). For chimerism controls, recipient and donor DNA samples were typed by a variable number of tandem repeats.

Five loci were studied, and an informative locus (33.6 in chromosome 1) was screened in posttransplant samples to quantify the percentage of donor cells. The patient has remained free from disease recurrence since her transplant up to the present time, and her graft has been considered stable with 100 percent of her peripheral blood DNA of donor origin. ABO blood grouping after HSCT presented the donor group (O, D+), and no mixed field was observed in all of the tests carried out after day +89 posttransplantation.

Ten years after undergoing HSCT, she became pregnant naturally at 35 years, and prenatal examinations were collected only at 20 weeks of gestation. ABO blood group tests presented a characteristic mixed field of O and B red blood

cells (RBCs), observed clearly at 30 weeks of gestation until delivery. The chimerism analysis carried out during the same period of pregnancy showed 100 percent of donor origin. At 36 weeks of gestation, she had a placental abruption and delivered a normal preterm neonate (cesarean section). There were no other complications during pregnancy and no evidence of aplastic anemia recurrence. Six months after delivery, the donor ABO group was recovered (group O and no mixed field).

This case suggests that the transitory erythrocyte split chimerism was an epiphenomenon associated with pregnancy.

Erythroid chimerism was well described after HSCT for hemoglobinopathies (thalassemia and sickle cell disease),²⁻⁴ and type 1 regulatory T cells (T_{regs}) were implicated in the sustained long-term tolerance of these patients.^{5,6} T_{regs} limit immune responses and have been implicated in maternal tolerance of the fetus. Although in gestation, the peak in T_{regs} in peripheral blood lymphocytes occurs during the second trimester,⁷⁻⁹ our patient had a mixed field of O and B RBCs at 30 weeks, and we speculated that the mixed field could be associated with a T_{reg} deficiency.⁸ Unfortunately, we did not analyze the T_{reg} population, which should be addressed in another similar situation. The biologic mechanisms underlying these observations require further investigation and would contribute toward the knowledge of the real risks involved in disease relapse and pregnancy complications after transplantations.

M.L. Barjas-Castro, MD, PhD

A.C. Vigoritto, MD, PhD

F.A. Moretto

V. Castro, MD, PhD

Hematology and Transfusion Center

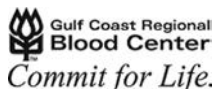
University of Campinas-UNICAMP

Campinas, São Paulo, Brazil

References

1. Au WY, Leung WC. Challenges and pitfalls in prenatal screening in pregnancies involving allogeneic stem cell transplantation. *Bone Marrow Transplant* 2007;39:379–82.
2. Andreani M, Manna M, Lucarelli G, et al. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood* 1996;87:3494–9.
3. Andreani M, Testi M, Battarra M, Lucarelli G. Split chimerism between nucleated and red blood cells after bone marrow transplantation for haemoglobinopathies. *Chimerism* 2011;2: 21–2.
4. Andreani M, Testi M, Gaziev J, et al. Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. *Haematologica* 2011;96:128–33.
5. Kurtz J, Wekerle T, Sykes M. Tolerance in mixed chimerism—a role for regulatory cells? *Trends Immunol* 2004;25:518–23.
6. Serafini G, Andreani M, Testi M, et al. Type 1 regulatory T cells are associated with persistent split erythroid/lymphoid chimerism after allogeneic hematopoietic stem cell transplantation for thalassemia. *Haematologica* 2009;94: 1415–26.
7. Mjösberg J, Svensson J, Johansson E, et al. Systemic reduction of suppressive CD4^{dim}CD25^{high}Foxp3+ T_{regs} in human second trimester pregnancy is induced by progesterone and 17 β -estradiol. *J Immunol* 2009;183:759–69.
8. Kahn DA, Baltimore D. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. *Proc Natl Acad Sci U S A* 2010;107:9299–304.
9. Chen SJ, Liu YL, Sytwu HK. Immunologic regulation in pregnancy: from mechanism to therapeutic strategy for immunomodulation. *Clin Dev Immunol* 2012;2012:258391.

SBB Last Chance Review: February 14 and 15, 2015 (Saturday and Sunday), onsite or by Webinar



Sponsored by Gulf Coast Regional Blood Center in Houston, this intensive, 2-day annual blood banking review is designed to benefit individuals preparing to take the ASCP Board of Certification examination in the SBB or BB category, physicians preparing for the board examination in blood banking, as well as individuals seeking a refresher course in blood banking. This SBB review provides 13 P.A.C.E. California and Florida continuing education hours. Included in the registration are handouts with references, case studies, and practice questions. Registration details at <http://www.giveblood.org/education/sbb-last-chance-review/>. Contact Clare Wong, 713-791-6201, cwong@giveblood.org.

Manuscripts

The editorial staff of *Immunohematology* welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. For instructions for scientific articles, case reports,

and review articles, see Instructions for Authors in every issue of *Immunohematology* or e-mail a request to immuno@redcross.org. **Include fax and phone numbers and e-mail address with all manuscripts and correspondence.** E-mail all manuscripts to immuno@redcross.org

Free Classified Ads and Announcements

Immunohematology will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) **without charge.**

E-mail information to immuno@redcross.org or fax to (215) 451-2538



The Johns Hopkins Hospital Specialist in Blood Bank Technology Program

The Johns Hopkins Hospital was founded in 1889. It is located in Baltimore, Maryland, on the original founding site, just 45 minutes from Washington, DC. There are approximately 1,000 inpatient beds and another 1,200 outpatient visits daily; nearly 600,000 patients are treated each year.

The Johns Hopkins Hospital Transfusion Medicine Division is one of the busiest in the country and can provide opportunities to perform tasks that represent the entire spectrum of Immunohematology and Transfusion Medicine practice. It provides comprehensive support to all routine and specialized areas of care for surgery, oncology, cardiac, obstetrics, neonatal and pediatric, solid organ and bone marrow transplant, therapeutic apheresis, and patients with hematological disorders to name a few. Our intradepartment Immunohematology Reference Laboratory provides resolution of complex serologic problems, transfusion management, platelet antibody, and molecular genotype testing.

The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is an onsite work-study, graduate-level training program for certified Medical Technologists, Medical Laboratory Scientists, and Technologists in Blood Banking with at least two years of full-time Blood Bank experience.

The variety of patients, the size, and the general intellectual environment of the hospital provide excellent opportunities for training in Blood Banking. It is a challenging program that will prepare competent and knowledgeable graduates who will be able to effectively apply practical and theoretical skills in a variety of employment settings. The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP). Please visit our website at <http://pathology.jhu.edu/departments/divisions/transfusion/sbb.cfm> for additional information.

Contact: Lorraine N. Blagg, MA, MLS(ASCP)CMSBB
Program Director
E-mail: lblagg1@jhmi.edu
Phone: (410) 502-9584

The Johns Hopkins Hospital
Department of Pathology
Division of Transfusion Medicine
Sheikh Zayed Tower, Room 3100
1800 Orleans Street
Baltimore, Maryland 21287

Phone (410) 955-6580
Fax (410) 955-0618
Web site: <http://pathology.jhu.edu/departments/divisions/transfusion/index.cfm>



Masters (MSc) in Transfusion and Transplantation Sciences at The University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2014 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following the MSc. The syllabus is organized jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

- Scientific principles of transfusion and transplantation
- Clinical applications of these principles
- Practical techniques in transfusion and transplantation
- Principles of study design and biostatistics
- An original research project

Application can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:

<http://ibgrl.blood.co.uk/MSc/MscHome.htm>

For further details and application forms please **contact**:

Dr Patricia Denning-Kendall

University of Bristol

Paul O'Gorman Lifeline Centre

Department of Pathology and Microbiology

Southmead Hospital

Westbury-on-Trym, Bristol BS10 5NB, England

Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: p.a.denning-kendall@bristol.ac.uk.

Online Specialist in Blood Bank (SBB) Certificate and Masters in Clinical Laboratory Management Program Rush University College of Health Sciences



Continue to work and earn graduate credit while the Rush University SBB/MS program prepares you for the SBB exam and the Diplomat in Laboratory Management (DLM) exam given by ASCP Board of Certification! (Please note acceptable clinical experience is required for these exams.)

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 2014. For additional information and requirements, please visit our website at: www.rushu.rush.edu/cls/

Rush University is fully accredited by the Higher Learning Commission [HLC] of the North Central Association of Colleges and Schools and the SBB Certificate Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP).

Applications for the SBB/MS Program can be submitted online at the following website:
<http://www.rushu.rush.edu/admiss/hlthadm.html>

Contact: Yolanda Sanchez, MS, MLS(ASCP)^{CM}SBB, Director, by email at Yolanda_Sanchez@rush.edu or by phone at 312-942-2402 or Denise Harmening, PhD, MT(ASCP), Director of Curriculum by e-mail at Denise_Harmening@rush.edu



Reference and Consultation Services

Antibody identification and problem resolution

HLA-A, B, C, and DR typing
HLA-disease association typing
Paternity testing/DNA

FOR INFORMATION, CONTACT:

Mehdizadeh Kashi
at (503) 280-0210

or write to:

Tissue Typing Laboratory
American Red Cross Biomedical Services
Pacific Northwest Region
3131 North Vancouver
Portland, OR 97227



American
Red Cross

CLIA licensed, ASHI accredited

IgA/Anti-IgA Testing

IgA and anti-IgA testing are available to do the following:

- Identify IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

Our ELISA for IgA detects protein to 0.05 mg/dL.

FOR ADDITIONAL INFORMATION CONTACT:

Cynthia Flickinger at (215) 451-4909

or e-mail:

Cynthia.Flickinger@redcross.org

or write to:

American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Cynthia Flickinger



American
Red Cross

CLIA licensed

National Reference Laboratory for Blood Group Serology

Immunohematology Reference Laboratory

AABB, ARC, New York State, and CLIA licensed

24-HOUR PHONE NUMBER:

(215) 451-4901

FAX: (215) 451-2538

American Rare Donor Program

24-HOUR PHONE NUMBER:

(215) 451-4900

FAX: (215) 451-2538

ardp@redcross.org

Immunohematology

PHONE, BUSINESS HOURS:

(215) 451-4902

FAX: (215) 451-2538

immuno@redcross.org

Quality Control of Cryoprecipitated-AHF

PHONE, BUSINESS HOURS:

(215) 451-4903

FAX: (215) 451-2538



American
Red Cross

Donor IgA Screening

- Effective tool for screening large volumes of donors
- Gel diffusion test that has a 15-year proven track record:
Approximately 90 percent of all donors identified as IgA deficient by this method are confirmed by the more sensitive testing methods

FOR ADDITIONAL INFORMATION:

Kathy Kaherl
at (860) 678-2764

e-mail:

Katherine.Kaherl@redcross.org

or write to:

Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Ave.
Farmington, CT 06032



American
Red Cross

CLIA licensed

National Platelet Serology Reference Laboratory

Diagnostic testing for:

- Neonatal alloimmune thrombocytopenia (NAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

Test methods:

- GTI systems tests
 - detection of glycoprotein-specific platelet antibodies
 - detection of heparin-induced antibodies (PF4 ELISA)
- Platelet suspension immunofluorescence test (PSIFT)
- Solid phase red cell adherence (SPRCA) assay
- Monoclonal immobilization of platelet antigens (MAIPA)
- Molecular analysis for HPA-1a/1b

FOR FURTHER INFORMATION, CONTACT:

Platelet Serology Laboratory (215) 451-4205

Cynthia Flickinger (215) 451-4909
Cynthia.Flickinger@redcross.org

Sandra Nance (215) 451-4362
Sandra.Nance@redcross.org



American
Red Cross

American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

CLIA licensed

National Neutrophil Serology Reference Laboratory

Our laboratory specializes in granulocyte antibody detection and granulocyte antigen typing.

Indications for granulocyte serology testing include:

- Alloimmune neonatal neutropenia (ANN)
- Autoimmune neutropenia (AIN)
- Transfusion-related acute lung injury (TRALI)

Methodologies employed:

- Granulocyte agglutination (GA)
- Granulocyte immunofluorescence by flow cytometry (GIF)
- Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:

- HLA (PRA) Class I and Class II antibody detection

FOR FURTHER INFORMATION, CONTACT:

Neutrophil Serology Laboratory (651) 291-6797

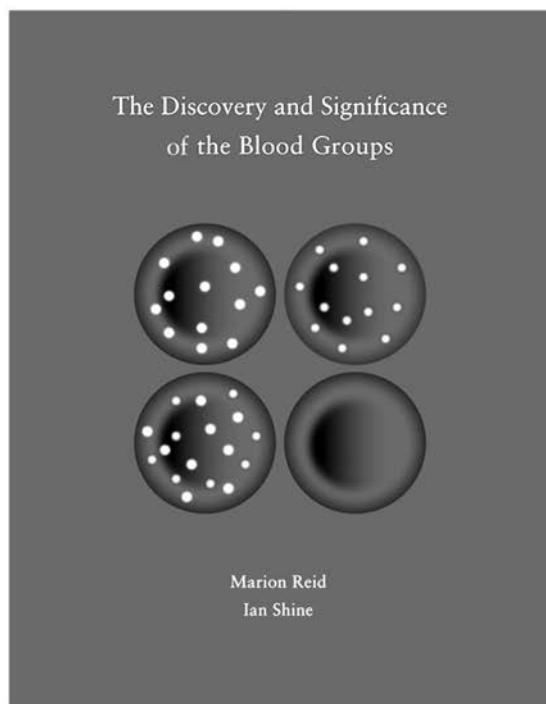
Randy Schuller (651) 291-6758
Randy.Schuller@redcross.org



American
Red Cross

American Red Cross Biomedical Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107

CLIA licensed



*A must for the bookshelf of every blood bank.
A superb and beautiful book.*

John Gorman MD,
Lasker Award 1980

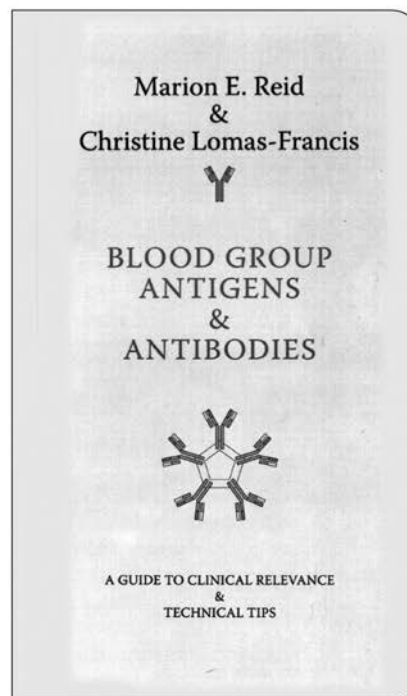
*The authors convey the excitement of scientific
discovery so effectively on every page.*

S. Gerald Sandler, MD
Professor of Medicine and Pathology,
Georgetown University Hospital

*It's more like reading an eye-witness account
than a scientific textbook. Wonderfully readable,
and a great addition to the books on blood groups.*

Phyllis Walker, MT(ASCP)SBB,
San Francisco

The Discovery and Significance of the Blood Groups
by Marion Reid & Ian Shine
Illus. / Hardcover / Full color / 7.5"x10" / 214 pp. / \$36.00
ISBN 978-1-59572-422-9



*A landmark book . . . Every MD and clinical
transfusion service should have their own
personal copy.*

Sandra J. Nance

*The most valued and useful resource in my
blood banking and immunohematology library
. . . it is highly readable and an enjoyable,
painless way to update your information about
blood group antigens.*

Immunohematology

Blood Group Antigens & Antibodies
by Marion Reid & Christine Lomas-Francis
Paperback / 5.4" x 8.4" / 214 pp. / \$25.00
ISBN 978-1-59572-103-7

AVAILABLE NOW!
To order, call 718-784-9112
or visit
www.bloodgroups.info
www.sbbpocketbook.com

SBB
BOOKS

Becoming a Specialist in Blood Banking (SBB)

What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) Board of Certification (BOC) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues preparing and implementing corrective actions to prevent and document nonconformances
- Design and present educational programs
- Provide technical and scientific training in transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services	Executives and Managers of Blood Centers	LIS Coordinators	Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers	
Quality Assurance Officers	Technical Representatives	Reference Lab Specialists	

Why become an SBB?

Professional growth	Job placement	Job satisfaction	Career advancement
---------------------	---------------	------------------	--------------------

How does one become an SBB?


















CAAHEP-accredited SBB Technology program or grandfather the exam based on ASCP education and experience criteria.

Fact: In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam compared to individuals who grandfather the exam. The **BEST** route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

Which approach are you more compatible with?

Contact the following programs for more information:

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

Program	Contact Name	Phone Contact	E-mail Contact	Web Site	Onsite or Online Program
Blood Systems Laboratories	Marie P. Holub	602-996-2396	mholub@bloodsystems.org	www.bloodsystemslaboratories.org	
Walter Reed Army Medical Center	William Turcan	301-295-8605	William.Turcan@med.navy.mil William.turcan@us.army.mil	www.militaryblood.dod.mil/Fellow/default.aspx	
American Red Cross, Southern California Region	Catherine Hernandez	909-859-7496	Catherine.Hernandez@redcross.org	www.redcrossblood.org/socal/communityeducation	
ARC-Central OH Region	Joanne Kosanke	614-253-2740 ext. 2270	Joanne.Kosanke@redcross.org	none	
Blood Center of Wisconsin	Phyllis Kirchner	414-937-6271	Phyllis.Kirchner@bcw.edu	www.bcw.edu	
Community Blood Center/CTS Dayton, Ohio	Nancy Lang	937-461-3293	nlang@cbccts.org	www.cbccts.org/education/sbb.htm	
Gulf Coast Regional Blood Center	Clare Wong	713-791-6201	cwong@giveblood.org	www.giveblood.org/services/education/sbb-distance-program	
Hoxworth Blood Center, University of Cincinnati Medical Center	Pamela English	513-558-1275	Inglishpf@ucmail.uc.edu	www.grad.uc.edu	
Indiana Blood Center	Jayanna Slayten	317-916-5186	jslayten@indianablood.org	www.indianablood.org	
Johns Hopkins Hospital	Lorraine N. Blagg	410-502-9584	lblagg1@jhmi.edu	http://pathology.jhu.edu/departments/divisions/transfusion/sbb.cfm	
Medical Center of Louisiana	Karen Kirkley	504-903-3954	kkirk1@lsuhsc.edu	www.mcino.org/webresources/index.html	
NIH Clinical Center Blood Bank	Karen Byrne	301-496-8335	Kbyrne@mail.cc.nih.gov	www.cc.nih.gov/dtm	
Rush University	Yolanda Sanchez	312-942-2402	Yolanda_Sanchez@rush.edu	www.rushu.rush.edu/cls	
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 ext. 1514	mdoty@fbsblood.org	www.fbsblood.org	
Univ. of Texas Health Science Center at San Antonio	Linda Myers	210-731-5526	lmyers@bloodntissue.org	www.sbbfosa.org	
University of Texas Medical Branch at Galveston	Janet Vincent	409-772-3055	jvincent@utmb.edu	www.utmb.edu/sbb	
University of Texas SW Medical Center	Lesley Lee	214-648-1785	lesley.lee@utsouthwestern.edu	www.utsouthwestern.edu/education/school-of-health-professions/programs/certificate-programs/medical-laboratory-sciences/index.html	

Revised May 2012

Immunohematology

Instructions for Authors

I. GENERAL INSTRUCTIONS

Before submitting a manuscript, consult current issues of *Immunohematology* for style.

Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW

A. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

B. Preparation of manuscript

1. Title page
 - a. Full title of manuscript with only first letter of first word capitalized (bold title)
 - b. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
 - c. Running title of ≤ 40 characters, including spaces
 - d. Three to ten key words
2. Abstract
 - a. One paragraph, no longer than 300 words
 - b. Purpose, methods, findings, and conclusion of study
3. Key words
 - a. List under abstract
4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
 - a. Introduction — Purpose and rationale for study, including pertinent background references
 - b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
 - c. Materials and Methods — Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient's names or hospital numbers.
 - d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
 - e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
5. Acknowledgments: Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
6. References
 - a. In text, use superscript, Arabic numbers.
 - b. Number references consecutively in the order they occur in the text.
7. Tables
 - a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of. . .) use no punctuation at the end of the title.

- b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.

- c. Place explanation in footnotes (sequence: *, †, ‡, §, ¶, **, ††).

8. Figures

- a. Figures can be submitted either by e-mail or as photographs (5 × 7" glossy).
- b. Place caption for a figure on a separate page (e.g. Fig. 1 Results of. . .), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.

- c. When plotting points on a figure, use the following symbols if possible:

○ ● △ ▲ □ ■.

9. Author information

- a. List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable. Provide e-mail addresses of all authors.

III. EDUCATIONAL FORUM

A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:

1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
2. Annotated conference proceedings

B. Preparation of manuscript

1. Title page
 - a. Capitalize first word of title.
 - b. Initials and last name of each author (no degrees; all CAPS)
2. Text
 - a. Case should be written as progressive disclosure and may include the following headings, as appropriate
 - i. Clinical Case Presentation: Clinical information and differential diagnosis
 - ii. Immunohematologic Evaluation and Results: Serology and molecular testing
 - iii. Interpretation: Include interpretation of laboratory results, correlating with clinical findings
 - iv. Recommended Therapy: Include both transfusion and nontransfusion-based therapies
 - v. Discussion: Brief review of literature with unique features of this case
 - vi. Reference: Limited to those directly pertinent
 - vii. Author information (see II.B.9.)
 - viii. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR

A. Preparation

1. Heading (To the Editor)
2. Title (first word capitalized)
3. Text (written in letter [paragraph] format)
4. Author(s) (type flush right; for first author: name, degree, institution, address [including city, state, Zip code and country]; for other authors: name, degree, institution, city and state)
5. References (limited to ten)
6. Table or figure (limited to one)

Send all manuscripts by e-mail to immuno@redcross.org

Immunohematology

Instructions for Authors | New Blood Group Allele Reports

A. For describing an allele which has not been described in a peer-reviewed publication and for which an allele name or provisional allele name has been assigned by the ISBT Working Party on Blood Group Allele Terminology (<http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/>)

B. Preparation

1. Title: Allele Name (Allele Detail)
ex. *RHCE*01.01 (RHCE*ce48C)*
2. Author Names (initials and last name of each (no degrees, ALL CAPS))

C. Text

1. Case Report
 - i. Clinical and immunohematologic data
 - ii. Race/ethnicity and country of origin of proband, if known
2. Materials and Methods
Description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient names or hospital numbers.
3. Results
Complete the Table Below:

Phenotype	Allele Name	Nucleotide(s)	Exon(s)	Amino Acid(s)	Allele Detail	References
e weak	<i>RHCE*01.01</i>	48G>C	1	Trp16Cys	<i>RHCE*ce48C</i>	1

Column 1: Describe the immunohematologic phenotype (ex. weak or negative for an antigen).

Column 2: List the allele name or provisional allele name.

Column 3: List the nucleotide number and the change, using the reference sequence (see ISBT Blood Group Allele Terminology Pages for reference sequence ID).

Column 4: List the exons where changes in nucleotide sequence were detected.

Column 5: List the amino acids that are predicted to be changed, using the three-letter amino acid code.

Column 6: List the non-consensus nucleotides after the gene name and asterisk.

Column 7: If this allele was described in a meeting abstract, please assign a reference number and list in the Reference section.

4. Additional Information
 - i. Indicate whether the variant is listed in the dbSNP database (<http://www.ncbi.nlm.nih.gov/snp/>); if so, provide rs number and any population frequency information, if available.
 - ii. Indicate whether the authors performed any population screening and if so, what the allele and genotype frequencies were.
 - iii. Indicate whether the authors developed a genotyping assay to screen for this variant and if so, describe in detail here.
 - iv. Indicate whether this variant was found associated with other variants already reported (ex. *RHCE*ce48C,1025T* is often linked to *RHD*DI/a-2*)

D. Acknowledgments

E. References

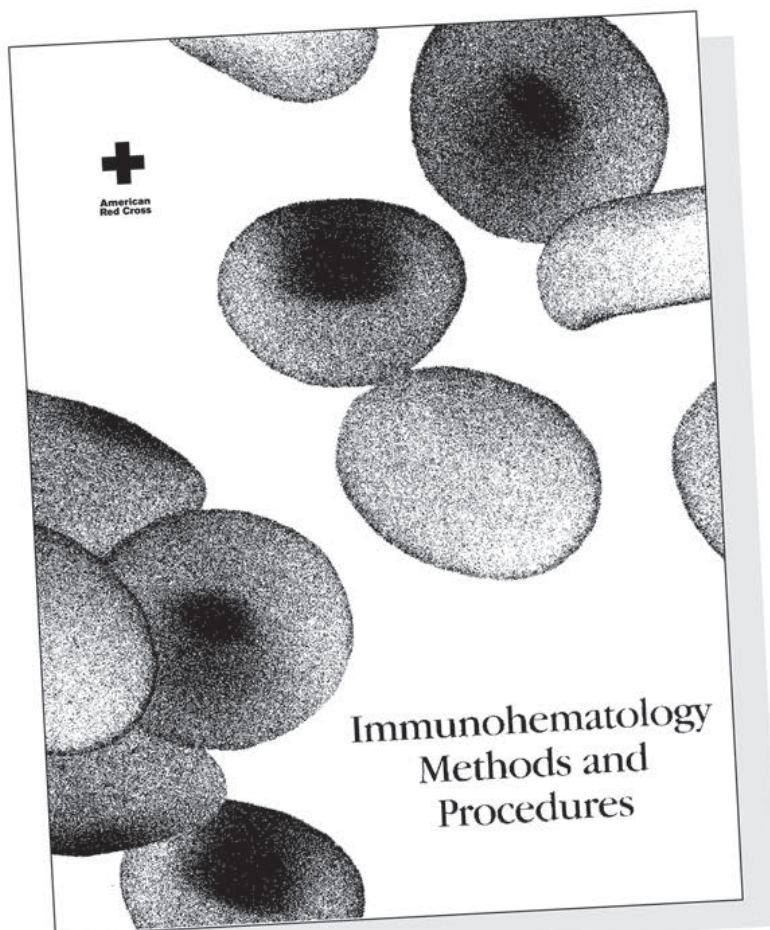
F. Author Information

List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable.

From the publishers of *Immunohematology*

A

***Comprehensive
Laboratory
Manual***



Featuring—

- Over 100 methods—
just about every method used in a reference lab.
- Eleven chapters discussing problems faced by blood group serologists and the procedures and methods that can be used to solve them.
- An extra set of the methods to use at the bench, printed on durable waterproof paper.
- See business reply order card enclosed in this issue or order on the Web at redcross.org/immunohematology



**American
Red Cross**

Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

NON PROFIT
U.S. POSTAGE
PAID
AMERICAN
RED CROSS

(Place Label Here)