

Elution Techniques in Blood Bank

George H. Roberts

Introduction

There are some terms used in association with elution that individuals must be able to define and understand. These terms include adsorption, elution, and absorption. Adsorption is the uptake of antibody by cells. Elution is the removal of antibody from cells. Absorption is the removal of antibody from serum, usually by adsorption.

Absorption techniques have a number of uses in blood bank including 1) separating a mixture of antibodies, 2) removing an autoantibody in order to detect presence of concomitant alloantibody, 3) removing an unwanted antibody, 4) confirming presence of specific antigens on red cell membrane, and 5) confirming specificity of antibody.

Elution is the process of removing antibodies from the surface of red blood cells. This can be accomplished by a variety of techniques that will be discussed below.

Uses of Elution Techniques

Prior to performing an elution, a direct antiglobulin profile (DAT) should be performed on the patient's red blood cells. If the DAT is negative or positive with complement only, the likelihood of retrieving an antibody from the red cells is not likely. Thus, performing an elution on cells with a negative DAT or sensitized with complement only is a waste of time, effort, and money in most instances. When the DAT is positive with IgG antibody or IgG antibody and complement, an elution may provide significant results. An elution may be indicated to aid in the 1) diagnosis of autoimmune hemolytic anemia (AIHA), 2) diagnosis ABO hemolytic disease of the newborn (HDN), 3) identification of specificity when multiple antibodies exist in a patient's serum or plasma, and 4) phenotyping red cells in patients with a positive DAT.

Diagnosis of Autoimmune Hemolytic Anemia (AIHA)

Although the DAT provides useful information, the definitive diagnosis of AIHA is dependent upon the characterization of the antibodies present in the patient's serum and in the red cell eluate. If the DAT is positive, it is essential to remove the antibody from the cells and determine its specificity.

Occasionally, red cells with a negative antiglobulin test will provide a strongly reactive eluate. The antibodies that perform in this manner usually belong to blood groups other than Rh.

A positive DAT with polyspecific AHG and

monospecific C3 indicates that the cells are coated with complement components only. Therefore, the eluate will produce negative results. These results could indicate the presence of a cold-reacting alloantibody that fixes complement, a cold-reacting autoantibody that fixes complement, or that complement is being fixed by the alternate or properdin pathway of complement activation.

If the DAT is positive with monospecific IgG AHG reagent, IgG antibody is sensitizing the red cells. This IgG antibody can be eluted from the red cells and possibly identified by testing the eluate against a panel of phenotyped red cells. If the DAT is positive due to IgG sensitization, yet the eluate shows no reactivity against normal phenotyped RBCs, drug-induced AIHA can be suspected or the antibody may be against a low incidence antigen. This situation will require other techniques in order to assign a specificity to the involved antibody such as 1) investigating patient medication history to determine medications that could be involved and following up with appropriate testing with medication-coated red cells, and 2) reacting mother's serum or baby's eluate with father's red cells to detect antibody to familial or low incidence antigens causing HDN.

Diagnosis of ABO Hemolytic Disease of the Newborn (HDN)

One of the best diagnostic tests for ABO HDN is to prepare an eluate from the infant's red cells and test that eluate against adult A and B cells because the DAT in ABO HDN may be weakly positive or even negative. The eluate will be negative with group O red cells if the antibody(ies) involved are a part of the ABO blood group system.

Identification of Specificity when Multiple Antibodies Are Present

When multiple antibodies are encountered, selective absorption and elution may be used to separate the mixture of antibodies. For example, initial antibody screening results indicate the probable presence of anti-C, anti-K, and anti-Jkb. The anti-C could be removed from the patient's serum by absorbing the antibody onto cells that are phenotypically big C positive, K negative, and Jkb negative. This would remove the big C antibody which could then be eluted and identified in the eluate. A second adsorption could then be performed on the absorbed serum/plasma using K negative, Jkb positive red cells to remove the Jkb antibody and leave the K in the serum. In

*George H. Roberts,
EdD, MT(AMT),
Professor and Department Head, Clinical Laboratory Science,
The University of Louisiana at Monroe*

Elution Techniques

TECHNIQUE	PROCEDURE	ACTION	ADVANTAGES/ DISADVANTAGES	USES
Temperature Landsteiner Heat	Heat at 56°C with agitation. Spin and harvest eluate	Heat causes thermal dissociation of AgAb complex and denatures cell membrane	Rapid and easy. Eluate stained with hemoglobin. Less effective for warm auto/alloantibodies	ABO antibodies
Lui Freeze-Thaw	Rapid freeze at -30°C or lower. Rapid thaw at 37°C. Spin and harvest eluate	Rapid extremes in temperature cause the dissociation by change in red cell membrane structural configuration	Same as heat	ABO antibodies
Ultrasonic	Washed RBC in ultrasonic bath one minute, spin and harvest eluate	Dissociation by shearing forces from high frequency sound waves and thermal energy produced by sonication	Same as heat but needs special sonication device	ABO antibodies
Acid Elution Digitonin-Acid	Digitonin lysis of RBC. Wash yields a hemoglobin-free stroma. Add glycine buffer (pH 3.0) to stroma. Readjust pH with phosphate buffer after elution	Acid alters charge of proteins causing a change in structural configuration	Clear eluate. Slow process requiring many washes	Most allo and autoantibodies. Decreased sensitivity for Kidd system
Cold Acid	Add cold (4°C) glycine (pH 3.0) directly to RBC. Incubate one minute at 4°C. Spin and harvest eluate. Readjust pH with phosphate buffer	Acid alters the charge of proteins causing a change in structural configuration	Requires less time than digitonin. Eluate stained with hemoglobin. Less sensitive in eluting many alloantibodies	Some alloantibodies and autoantibodies.
Organic Solvents Ether	Mix ether, saline, and RBCs one minute. Vent, spin, and harvest eluate. Incubate 37°C or 56°C to drive off residual ether	Denatures RBC membrane destroying structural conformation of lipid bilayer or may alter tertiary antibody structure	Highly flammable and explosive. Must store in explosion-proof refrigerator	Most alloantibodies and autoantibodies. Decreased sensitivity for Anti-S/s
Xylene	Mix cells and xylene and incubate at 56°C for 10 minutes. Remove xylene and stroma by vacuum aspiration. Harvest eluate	Same as ether	Flammable, but does not require explosion-proof refrigerator. Requires vacuum aspiration	Most alloantibodies and autoantibodies
Chloroform	Mix cells, albumin, and chloroform. Spin and harvest eluate.	Same as ether	Nonflammable, nonexplosive carcinogen	Most alloantibodies and autoantibodies
Procedures for antigen-typing DAT-positive RBC Modified Heat	Heat 45°C with frequent agitation	Heat causes thermal dissociation of AgAb complex without denaturing red cell membrane	Rapid and easy. Some antigens may be damaged. RBC remain intact	Preparing DAT-positive RBC for antigen typing
Chloroquine diphosphate	Incubate 1 volume cells and 4 volumes chloroquine diphosphate	Causes dissociation of immune complexes	Requires two hour incubation	Same as modified heat

some instances, rather than using multiple absorptions, the scientist may choose to use selected panel cells to demonstrate specificity in cases of multiple antibodies.

To accurately phenotype red cells with positive DAT

When cells are heavily coated with IgG antibodies, tests with antisera of high protein content are difficult and those with antiglobulin reactive antisera are impossible. It is necessary to dissociate antibody from

the cell surface without damaging red cell integrity or altering antigenic reactivity. The desired end product is the red cell, not the antibody. As an example, in Rh and other forms of HDN (those due to antibodies other than Rh), the DAT may be positive due to blocking of the antigen sites by high titers of antibody. For example, Rh positive red cells giving a strongly positive DAT may type as Rh negative due to the blocking of Rh receptors. If an attempt is made to perform a weak D test, the test and the associated Rh control will both be positive. This fact can be a seri-

ous pitfall for the novice blood banker. Note that the D antibody is capable of causing this reaction. To overcome this problem, a gentle heat elution or the chloroquine diphosphate elution can be used since neither of these procedures disrupts the red cells but leaves them intact where appropriate antigen typing procedures can be performed (see table on previous page).

Comments on Elution

Elution removes antibody molecules from the red cell membrane either by disrupting the antigen or changing conditions to favor dissociation of antibody from antigen. Many techniques are available, and no single method is best in all situations. If an eluate prepared by one technique is unsatisfactory, it may be helpful to prepare another eluate utilizing a different technique.

The red cells used for any elution technique must be thoroughly washed to remove all antibody except that bound to the cells. Six washes with large volumes of saline is usually sufficient. Adequacy of washing is tested by examining saline from the last wash for the presence of antibody by the indirect antiglobulin (IAT) procedure. If antibody is detectable in the last wash, there could be enough unbound antibody molecules still present so that results obtained on testing the eluate are not valid. This assumes the possible mixture of alloantibody and autoantibody.

As soon as the elution is completed, remove the supernatant fluid and place it into a separate tube to avoid reattachment of antibody to cell stroma and a possible false negative test result.

Elution Techniques

There are several different techniques for elution based upon the process used to cause dissociation of the antigen/antibody complex. The table below indicates the various techniques used, describes the procedure and action, lists the advantages and disadvantages of each, and gives times when each method might be appropriate to use.

Suggested Readings

Blaney, K, & Howard, P. (2000). *Basic and Applied Concepts of Immunohematology*. St. Louis: Mosby.

Brecher, M. (2002) *American Association of Blood Banks Technical Manual*, 14th edition. Bethesda: AABB.

Harmening, D. (2005). *Modern Blood Banking and Transfusion Practices*, 5th edition. Philadelphia: F. A. Davis Company.

Quinley, E. (1998). *Immunohematology Principles and Practice*. Philadelphia: Lippincott.

Rudmann, S. (1995). *Textbook of Blood Banking and Transfusion Medicine*. Philadelphia: W. B. Saunders Company.

Turgeon, M. (1995). *Fundamentals of Immunohematology*. Baltimore: Williams and Wilkins.

Questions for STEP Participants

Article **301**
0.1 CEC

Answer questions only on the official STEP answer sheet. If you do not have the official STEP answer sheet, a year's supply can be obtained (at no cost), simply by writing to: STEP Program Answer Sheets, American Medical Technologists, 710 Higgins Road, Park Ridge, IL 60068-5765, or by fax: 847/823-0458, or by e-mail: paula.simoncini@amt1.com.

In addition to marking your answers, be sure to include all the required information on the answer sheet and a processing fee of \$3.00 per article.

In the following, choose the *one* best answer for each question.

- 1** Removal of antibody from cells is a definition of:
 - A.** elution
 - B.** adsorption
 - C.** absorption
- 2** Removal of antibody from serum is a definition of
 - A.** elution
 - B.** absorption
 - C.** adsorption
- 3** The elution procedure would be of LEAST benefit in the determination of:
 - A.** diagnosis of ABO HDN
 - B.** characterization of AIHA
 - C.** single alloantibody identification
- 4** When the DAT is positive due to IgG sensitization and the eluate shows no reactivity, the first thing the scientist should do would be to:
 - A.** repeat elution with another procedure
 - B.** consider this an inconclusive finding
 - C.** tell physician that it is a drug-induced problem
- 5** Which of the following elution procedures will leave the red cells intact so that they can be antigen typed?
 - A.** Lui Easy Freeze
 - B.** Chloroquine diphosphate
 - C.** Landsteiner Heat Elution
- 6** The purpose for testing the sixth wash following an elution is to assure that all:
 - A.** complement is removed from sample
 - B.** bound antibody molecules are removed from sample
 - C.** unbound antibody molecules are removed from sample
- 7** Which of the following is NOT a basic principle underlying a particular elution technique?
 - A.** Dissociation based upon pH change
 - B.** Dissociation based upon temperature change
 - C.** Dissociation based upon increased time interval
- 8** Which of the following elution techniques is based upon physical motion to dissociate antibody from antigen?
 - A.** Ultrasonic
 - B.** Organic acids
 - C.** Digitonin-acid
- 9** What results would you expect on an eluate that had been prepared and allowed to remain in contact with the RBC for an extended period of time?.
 - A.** False negative
 - B.** False positive
 - C.** No observable effect
- 10** A group A Rh negative weak D negative mother with an anti-D titered at 1024 gave birth to a baby with the following laboratory results:
 - Baby blood type is group A Rh negative
 - DAT 4+ with polyspecific and monospecific IgG
 - Bilirubin 18.5 mg/dL (0.2-1.0).What is the MOST likely cause of this problem?
 - A.** Rh HDN
 - B.** ABO HDN
 - C.** Another type of HDN