TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	11.9.2012
Department:	Blood Bank	**Due Date:**	11.30.2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Acid Elution
Description of change(s):
• Initial volume of PACKED red cells to be washed was changed from 30 to 20 drops. The change was made to ensure we have at least 20 drops of WASHED red cells to use for eluate preparation. Fewer drops may be used when specimen volume is a problem.

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	**Signature**	**Date**

Technical SOP

Title	**Acid Elution**		
Prepared by	Stephanie Codina	Date:	7/12/2010
Owner	Stephanie Codina	Date:	7/12/2010

Laboratory Approval	**Local Effective Date:**		
Print Name	**Signature**		**Date**
Refer to the electronic signature page for approval and approval dates.			

Annual Review			
Print Name	**Signature**		**Date**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Acid Elution	ELU-KIT II	%ELU

Synonyms/Abbreviations
Eluate

Department
Blood Bank

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2. ANALYTICAL PRINCIPLE

Red blood cells coated with antibody are washed with a special wash solution to remove any unabsorbed antibody and to maintain the association of bound antibody. The washed cells are then suspended in a glycine buffer solution at low pH to dissociate the bound antibody. The recovered eluate is neutralized by adding a buffering solution. The eluate is then ready to be tested for antibody detection and/or identification.

3. SPECIMEN REQUIREMENTS

Refer to procedure 'Sample Specifications for Blood Bank Testing' for patient preparation and specimen type and handling.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Gamma ELU-KIT II	Immucor, Cat. #7861
Anti-IgG	Immucor, Cat.# 409210 or equivalent
Reagent red blood cells, 2-4%	Immucor 2381 (Panoscreen), 3032 (Panocell 10), 2332 (Panocell 16), 5020 (Panocell 20), or equivalent
Check Cells	Immucor, Cat. #2225 or equivalent

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Assay Kit	
Reagent a	Concentrated Wash Solution
Reagent b	Eluting Solution
Reagent c	Buffering Solution
Storage	Room temperature (15 – 30°C)

Stability	Up to expiration date. Do not use if markedly turbid.
Preparation	Ready for use except for Concentrated Wash Solution.

Reagent	Concentrated Wash Solution
Storage	1 – 8°C
Stability	Once prepared, the Working Wash Solution may be stored in a covered container (properly labeled) for 6 months at 1-8° C, do not use if turbid.
Preparation	Dilute 1 in 10 with laboratory reagent-grade water, place in a dispensing (squirt) bottle.

Reagent	Anti-IgG
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Ready to use as supplied.

Reagent	Panoscreen, Panocell 10, Panocell 16, or Panocell 20
Container	3ml each vial
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Resuspend red cells before use by gently inverting each vial several times.

Reagent	Coombs Control Cells (IgG Coated)
Container	10ml each
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Resuspend red cells before use by gently inverting each vial several times.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

A sample from the final wash is tested for antibody activity in parallel with the eluate to assure that any antibody present has been derived from the bound state on the original cells, and is not merely remaining as a result of inadequate washing.

All negative antiglobulin tests are confirmed with IgG-sensitized cells.

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

N/A

6.3 Frequency

With each test performed.

6.4 Tolerance Limits

Last wash testing should be negative. If antibody activity is present in the last wash, the elution procedure must be repeated after more thorough washing of the cells.

IgG coated Control Cells: Agglutination must be present at strength of 2+ or greater or the test results are invalid and the entire test must be repeated.

6.5 Review Patient Data

N/A

6.6 Documentation

Results are recorded on appropriate worksheet.

6.7 Quality Assurance Program

Participation in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Equipment

37°C dry heat incubator
Cell washer
Serological centrifuge
Agglutination viewer

7.2 Supplies

Test tubes, (12 x 75 mm)
Pipettes
Saline, 0.9%
Reagent-grade water

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included in the appropriate notebook/file.

All parts of this kit should be used together. Reagents from different Elu-Kit II boxes should not be mixed.

8.1 Wash the Red Cells

Step	Action
1	Prepare the working wash solution if needed. A. Add 1 volume of "concentrated wash solution" to 9 volumes of laboratory, reagent-grade water (i.e., add 5 mL of concentrated wash solution to 45 mL of water). B. Mix the working wash solution well. C. At a minimum, label the working wash solution with the following: a. Reagent name (working wash solution) b. Amount prepared c. Procedure (acid eluate) d. Date prepared e. Tech f. Expiration date (6 months from date prepared) g. Lot number h. Storage specifications (1-8°C, do not use if turbid)
2	Centrifuge the patient specimen per procedure, "Sample Specifications for Blood Bank Testing."

Step	Action
3	Label a test tube with the following information. Use of a pre-printed label is preferred. A. Patient's full name B. Patient's medical record number C. Specimen accession number D. Tech initials Remove as much serum/plasma as possible from the primary specimen tube and place it in the labeled aliquot tube. Place a cap on the serum/plasma so it does not spill.
4	Label a 12 x 75 mm test tube (DO NOT use a 10 x 75 mm test tube) with the patient identifiers. At a minimum, this will be the patient's initials or the first 3 letters of the patient's last name.
5	Place ≥30 drops of packed patient cells into the labeled test tube. It is acceptable to use a smaller volume of red cells if the sample volume is too low to obtain 30 drops. However, the volume of eluate prepared will be smaller also.
6	Wash the cells once with saline. Remove as much supernatant as possible. Do NOT use an automated cell washer for this step.
7	Wash the cells a minimum of 3 additional times using working wash solution. Using cold working wash solution (1-10°C) will minimize dissociation of the antibody from the cells during the wash phase. A. Fill the tube containing the red cells approximately 2/3 full with working wash solution. B. Cover the tube and invert until completely mixed. C. Serofuge the tube at the wash phase listed on the serofuge (generally 60 seconds). D. Remove all of the supernatant from the tube and discard. **Do not discard the supernatant after the 4th wash.**
7	Label a tube with the patient identifiers and "LW." Pipette the supernatant from the 4th wash into the test tube and cover until the eluate is complete.

8.2 Prepare the Eluate

Step	Action
1	Label a tube with the patient identifiers and "ELU."
2	Place 20 drops of washed red cells into the labeled tube. If fewer than 20 drops are used, remember the number of drops that are added so an equal volume of eluting solution can be added in the next step. **You must recount the number of drops of cells following the wash procedure. Some cells are lost during the wash.**

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Step	Action
3	Add 20 drops (or a number of drops that equals the number of drops of washed red cells in the tube) of eluting solution to the red cells.
4	Quickly but gently invert the tube 4 times to mix and serofuge for the wash phase listed on the serofuge. Prolonged immersion of the cells in eluting solution causes hemolysis and the consequent release of hemoglobin into the eluate alters the pH. This may affect the volume of buffering solution needed to adjust the pH to neutral.
5	Label a test tube with the patient identifiers and "ELU." Immediately transfer the supernatant to the clean test tube. Discard the deposited red cells as they are no longer suitable for testing.
6	Add sufficient buffering solution to restore the pH of the eluate to an acceptable range for testing (6.4 to 7.6). A. Aspirate buffering solution with a pipette and observe for a blue color. Do not use the buffering solution if the blue color is not present. B. Add buffering solution to the eluate dropwise and mix after each drop. Continue adding buffer until a blue color persists in the eluate following the mixing process.
7	Mix the eluate well and serofuge for the wash time listed on the front of the serofuge to remove any cellular debris.
8	Label a clean test tube with the patient identifiers and "ELU." Pipette the eluate into the clean test tube and discard any left over debris.

8.3 Testing the Eluate and Last Wash

Step	Action
1	Label 10 test tubes with the patient identifiers and the test contents. A. Label 5 of the tubes for the test cells and last wash fluid. a. Label 1 tube "LWI" or "LW1." b. Label 1 tube "LWII" or "LW2." c. Label 1 tube "LWIII" or "LW3." d. Label 1 tube "LWa." e. Label 1 tube "LWb." B. Label 5 of the tubes for the test cells and the eluate fluid. a. Label 1 tube "ELUI" or "ELU1." b. Label 1 tube "ELUII" or "ELU2." c. Label 1 tube "ELUIII" or "ELU3." d. Label 1 tube "ELUa." e. Label 1 tube "ELUb."

Step	Action
	Note: The "a" and "b" tubes may be omitted if the patient has not been transfused with out of group plasma, platelets, or cryoprecipitate products within the previous 90 days for all specimens EXCEPT cord bloods.
2	Place 1 drop of the appropriate reagent red blood cell in each tube. A. Place 1 drop of screening cell 1 in the tube labeled "LWI" or "LW1" **and** the tube labeled "ELUI" or "ELU1." B. Place 1 drop of screening cell 2 in the tube labeled "LWII" or "LW2" **and** the tube labeled "ELUII" or "ELU2." C. Place 1 drop of screening cell 3 in the tube labeled "LWIII" or "LW3" **and** the tube labeled "ELUIII" or "ELU3." D. Place 1 drop of the A_1 cell 1 in the tube labeled "LWa" **and** the tube labeled "ELUa." E. Place 1 drop of the B cell 1 in the tube labeled "LWb" **and** the tube labeled "ELUb."
3	Wash the reagent red blood cells once in saline and decant to a dry button. An automated cell washer may be used for this step.
4	Add 2 drops of eluate to the dry cell button in each tube labeled "ELU" and gently mix each tube.
5	Add 2 drops of the last wash solution to each tube labeled "LW" and gently mix each tube.
6	Incubate both sets of tubes at 37±1°C for 10 minutes. Incubation may be extended up to a maximum of 30 minutes. Incubating for the upper end of the time limit may enhance reactivity.
7	Add 10 drops of working wash solution to each tube and mix thoroughly.
8	Centrifuge for the wash phase of testing listed on the front of the serofuge.
9	Decant the supernatant completely and blot the tubes dry.
10	Add 2 drops of anti-IgG to each tube and mix well.
11	Serofuge the tubes for the AHG phase listed on the front of the serofuge.
12	Resuspend tubes by gentle shaking and read macroscopically for agglutination using an agglutination viewer. Record results immediately.
13	Add 1 drop of check cells to each negative tube and mix gently.
14	Serofuge the tubes for the AHG phase listed on the front of the serofuge.

Step	Action
15	Resuspend tubes by gentle shaking and read macroscopically for agglutination using an agglutination viewer. Check cells should yield positive results at strength 2+ or greater. If no agglutination is observed or the reactivity is less than 2+, the test is invalid and must be repeated.
16	Each tube with an "LW" should yield a negative AHG result. This tube is used as a control to assure that antibody present in the eluate has been derived from a bound state on the original cells and is not merely remaining as a result of inadequate washing. **If any "LW" tube yields a positive AHG result, the elution must be repeated after more thorough washing of the red cells.**
17	If an antibody is detected in the eluate, an eluate antibody identification must be performed. A. Label tubes with the patient's initials or first 3 letters of last name. B. Label each tube with "ELU" and the number of the panel cell that will be tested in the tube. C. Test per "Testing the Eluate" section above, but use panel cells. D. The last wash does **not** need to be tested in parallel with the panel cells. E. Perform antibody rule out and antibody identification per procedure, "Antibody Identification." Consider a possible delayed serologic transfusion reaction if an antibody is identified in the eluate and the patient has been recently transfused. Refer to procedure, "Transfusion Reaction."

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

A reaction with the screening cells indicates that antibody has been recovered from the patient's cells. The absence of agglutination with screening cells indicates that no antibody has been recovered.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

N/A

11. EXPECTED VALUES

11.1 Reference Ranges

N/A

11.2 Critical Values

N/A

11.3 Alert Values

N/A

12. CLINICAL SIGNIFICANCE

A positive eluate may be indicative of Hemolytic Disease of the Newborn, delayed serologic or hemolytic transfusion reaction, autoimmune disease, or related to certain medications or drug therapies.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

The yield of antibody obtained upon elution is dependent on the following variable factors:

1. The amount of antibody bound to the cells.
2. The degree of dissociation of antibody that occurs during the washing procedures.
3. The degree of recombination of antibody that occurs before the eluate is separated from the cells.
4. The degree to which immunoglobulin is denatured by the low pH during dissociation.

Other factors to be considered are:

5. Red blood cells having a positive DAT attributed to complement alone will normally yield a negative eluate.
6. Incorrect restoration of pH in the eluate may cause hemolysis or may inhibit antibody reactivity in subsequent testing.
7. Cells from samples stored more than 72 hours may yield less potent eluates.
8. A false negative test may occur if the modified antiglobulin test procedure is used and the test red cells are not washed sufficiently prior to testing.
9. Use of the wash solution provided with the kit has been reported to give rise to the possibility of non-specific uptake of particularly strong antibody to antigen-negative red blood cells, leading to false-positive results.

10. Failure to develop a pale blue color when Buffering Solution is added to a freshly prepared eluate may indicate degradation of the Buffering Solution and the eluate should be discarded. Confirm that the Buffering Solution is blue prior to use and repeat eluate.

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

N/A

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental, Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.

16. RELATED DOCUMENTS

SOP: Sample Specifications for Blood Bank Testing
SOP: Direct Antiglobulin Test (DAT)
SOP: Antibody Identification

17. REFERENCES

1. Gamma Elu-Kit II Package Insert, IC3021-1, ImmucorGamma: Norcross, GA. Revised 10/2007.

18. REVISION HISTORY

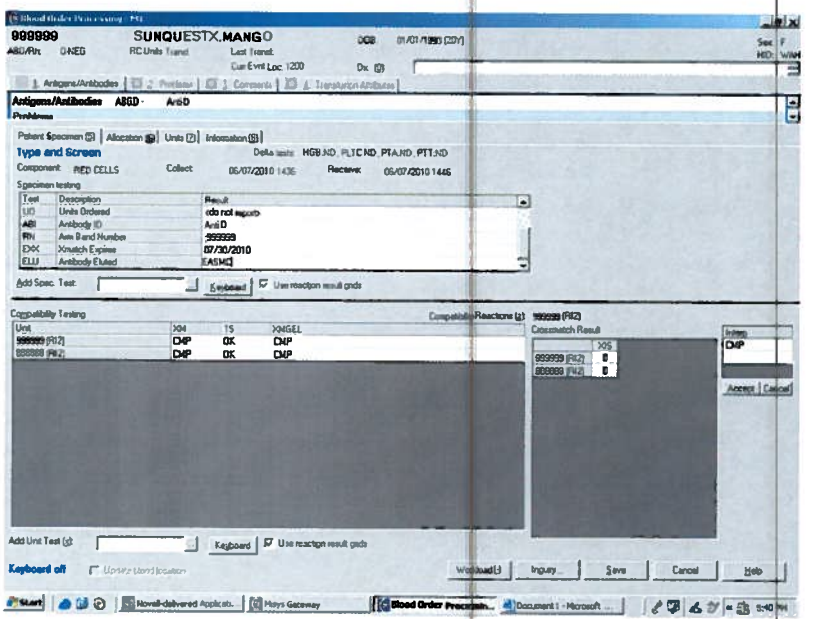
Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SWB.012.000		
000	9.15.11	8.3	Edited steps 4 & 5 for clarity.	SCodina	NCacciabeve
		8.2	Bolded requirement to recount RBC drops after wash for clarity.		
001	11.6.12	8.1	Updated procedure step 5 to instruct user to begin with a volume of ≥30 drops so there is sufficient sample after the wash.	SCodina	NCacciabeve

19. ADDENDA

A: Entering Eluate Results into the Sunquest LIS
B: Eluate Codes

Appendix A

Entering Eluate Results into the Sunquest LIS

Step	Action
1	Access the patient in Sunquest function, "Blood Order Processing" and open the T&S test.
2	In the "Add Spec Test" field, type "L" or ";ELU" and press the tab key. This will add the test. If an eluate was positive and an eluate panel was performed, you will also need to add the panel billing charges by typing "$" or ";PANEL" and pressing the tab key.
3	Enter the eluate result in the "ELU" field. A. Type "NEL" and press tab if the eluate yielded negative results. B. Type "PEL" and press tab if the eluate was positive on all cells tested. C. Type in the specific antibody identified if an antibody was identified in the eluate. Refer to appendix B for codes. 
4	If a panel charge was ordered, it will automatically result with the statement "Billed for services performed." If more than one eluate panel was performed, A. Highlight the result. B. Press the "Tab" key. A new result line will appear. C. Type semicolon (;) and the number of panels that should be billed to the patient then press the "tab" key.
5	Press the "save" button.

Appendix B

Eluate Codes

Code	Translation
EAA1	Anti-A1 eluted
EABGC	Anti-C eluted
EABGD	Anti-D eluted
EABGE	Anti-E eluted
EABGG	Anti-G eluted
EABGS	Anti-S eluted
EACEL	Anti-Cellano eluted
EADRH	Anti-D due to RHIG eluted
EAFYA	Anti-Fy(a) eluted
EAFYB	Anti- Fy(b) eluted
EAJKA	Anti-Jk(a) eluted
EAJKB	Anti-Jk(b) eluted
EAJSA	Anti-Js(a) eluted
EAJSB	Anti-Js(b) eluted
EAKEL	Anti-Kell eluted
EAKPA	Anti-Kp(a) eluted
EAKPB	Anti-Kp(b) eluted
EAM	Anti-M eluted
EAN	Anti-N eluted
EAP1	Anti-P1 eluted
EASMC	Anti-c eluted
EASME	Anti-e eluted
EASMS	Anti-s eluted
EAU	Anti-U
EAWIN	Anti-D due to Win Rho D eluted
EINCL	Inconclusive eluate
NEL	No antibody detected in eluate
PEL	Panagglutinin in eluate