	<b>Acid Elution</b>  BB.SP.1003.3	<b>Dept:</b>	324311
		<b>Dept Name</b>	Blood Bank
		<b>Effective Date:</b>	05/07/2001
		<b>Revised Date:</b>	
<b>Name &amp; Title:</b> CLIA Laboratory Medical Director		<b>Contact:</b>	Julie Simmons
<b>Signature:</b>		<b>Date:</b>	

## 1. General Procedure Statement:

**A. Purpose:** Acid Elution is used to aid in the identification of unexpected antibodies eluted from red cells. Unabsorbed antibody surrounding the sensitized red cells is removed by washing with a wash solution which prevents the loss of absorbed antibody from the red cells. After washing, the antigen-antibody complex is broken by addition of a low pH solution. The recovered eluate is buffered by adding a base buffering solution. The pH is checked and the eluate is then ready for use.

1. Eluates are prepared by acid elution to aid in the following:

- a) To identify the antibody responsible for a positive direct antiglobulin test in acquired hemolytic anemia or transfusion reaction.
- b) To demonstrate the presence of a weak antigen
- c) To identify antibodies causing hemolytic disease of the newborn.
- d) To prepare specific antibody from sera containing unwanted antibodies.

2 Acid elutions are to be performed when:

- a) Direct Coombs testing (DAT) is positive (DAT, C3d, IgG or IgG gel)
- b) DAT is negative but there is suspicion that offending antibody can be eluted (eg. Anti-Jka or Anti-Jkb)
- c) Requested by Physician or Management
- d) In correlation with other techniques (eq. absorption-elution)
- e) Remove antibody in eluate to use as future rare reagent.

## B. Responsible Department/Scope:

- i. Procedure owner/Implementer: Julie H. Simmons/Christina S. Warren
- ii. Procedure prepared by: Kate James
- iii. Who performs procedure: Department staff

**C. Definitions:**

- DI- deionized water
- Elu- elution
- LW- last wash
- DAT- direct antiglobulin test
- WK+- weakly positive
- LISS- low ionic strength medium
- PEG- polyethylene glycol
- SCC – Soft Computer Consultants, Blood Bank computer system

**D. Sections:**

- I. Preparation of Working Wash Solution
- II. Preparation of Acid Eluate
- III. Testing the Acid Eluate in Gel and Gel Ficin
- IV. Testing the Acid Eluate in LISS, PEG, Saline and Ficin Mediums
- V. QC of pH Paper

**2. Procedure: I. Preparation of Working Wash Solution**

Biological Risk Assessment: low  
 Chemical Risk Assessment: moderate  
 Protective Equipment: lab coat, gloves

**Supplies:** 12X75 test tubes, Transfer pipets: B/B-PET™, pH strips, Biohazard wipes

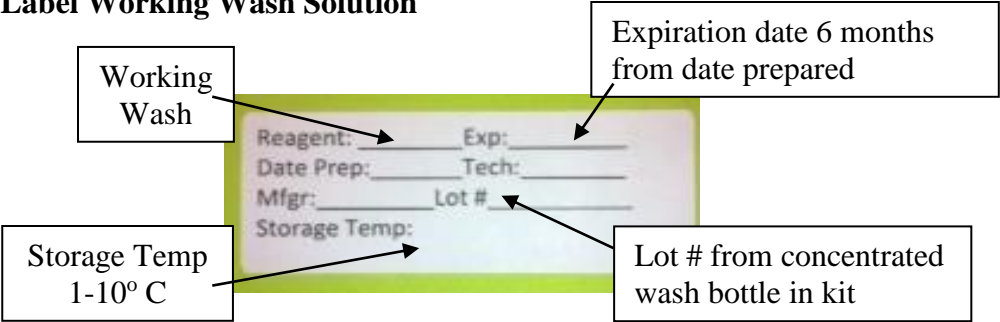
**Reagents:** 0.85% Blood Bank Saline, Immucor Gamma Elu-Kit™ II: consists of three solutions:  
 Concentrated Wash Solution, Eluting Solution and Buffering Solution: *These solutions may be interchanged between lot numbers, providing they are in date.*

**Equipment:** 500 ml graduated cylinder, 1000 ml flask, parafilm. polyethylene wash bottle.

**Specimen Requirements:** Pink or lavender top EDTA tube labeled with the patient’s name, medical record number, date of collection and identification of phlebotomist.

**Caution:** Concentrated Wash solution and Buffering Solution contain 0.1% sodium azide and is classified as harmful if swallowed. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
1.0	<p><b>Prepare Working Wash Solution</b></p> <p>1.1 Retrieve the Concentrated Wash Solution, the 50 ml bottle, from the kit.</p> <p>1.2 Measure 450 ml DI water in a 500 ml graduated cylinder.</p> <p style="padding-left: 20px;">a) Graduated cylinder is located behind the water baths in Component Prep area.</p> <p>1.3 Pour the 450 ml of DI water into a 1000 ml flask.</p> <p style="padding-left: 20px;">a) Flask is in old BMT tank room on top shelf of cabinet by the sink.</p>	

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
	1.4 Pour the entire bottle (50 ml) of concentrated wash solution into DI water. 1.5 Cover with Parafilm and mix well. 1.6 Store in a clean properly labeled polyethylene wash bottle.	
2.0	<p><b>Label Working Wash Solution</b></p>  <p>Blank Reagent Labels are located in the blood processing area in the drawer labeled: Blank Reagent Labels</p>	
3.0	<p><b>Return Working Wash Solution to refrigerator at 1°-6° C immediately after washing has been completed.</b></p>	
4.0	<p><b>Working Wash Solution will maintain a temperature of 1-10° C for 1 hour at room temperature.</b></p>	

## II. Preparation of Acid Eluate

Biological Risk Assessment: low  
 Chemical Risk Assessment: low  
 Protective Equipment: Lab Coat/Gloves

**Supplies:** 12X75 test tubes, Transfer pipets: B/B-PET™, pH strips, Biohazard wipes, Uni-Flex safety caps 12/13mm  
**Reagents:** 0.85% Blood Bank Saline, Immucor Gamma Elu-Kit™ II: consists of three solutions:

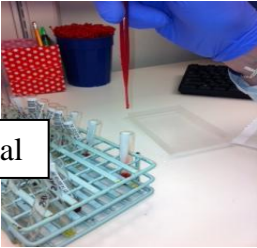

Concentrated Wash Solution, Eluting Solution and Buffering Solution: *These solutions may be interchanged between lot numbers, providing they are in date.*

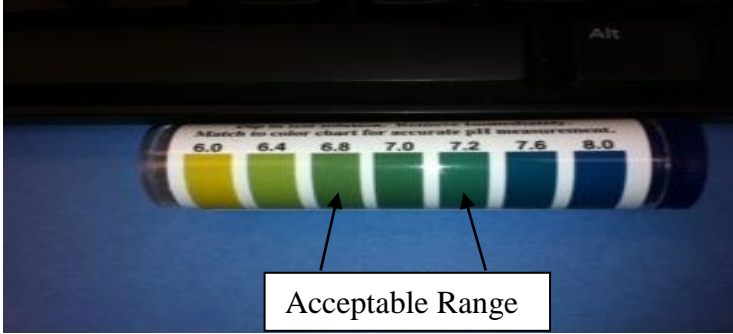
**Equipment:** high speed centrifuge, serofuge 3400-3600 RPM

**Specimen Requirements:** Pink or lavender top EDTA tube labeled with the patient's name, medical record number, date of collection and identification of phlebotomist.

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
1.0	<p><b>Label five 12X75mm test tubes as follows:</b></p> <p>1.1 Label <b>tube #1</b> with the first 3 initials of the patient's last name and mark a fill line near the top of the tube.</p> <p>1.2 Label <b>tubes 2&amp; 3</b> with the first 3 initials of the patient's last name and <u>elu</u>.</p> <p>1.3 Label <b>tube # 4</b> with a large patient label and <u>elution</u> written on the label or tube.</p> <p>1.4 Label <b>tube #5</b> with a small patient label and <u>LW</u> written on the label or tube.</p> <p>1.5 Refer to BB Policy.1001 Labeling Test Tubes</p> 	

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
2.0	<p><b>Centrifuge the EDTA specimen in the high speed centrifuge for 3 minutes and remove as much plasma as possible with a transfer pipet to a tube labeled with either a small or large patient label.</b></p>	
3.0	<p><b>Transfer red cells to tube #1.</b></p> <p>3.1 The volume of packed red cells should be at least 1ml or 20 drops.</p>	
4.0	<p><b>Washing Red Cells</b></p> <p>4.1 0.85% Isotonic Saline Wash</p> <ol style="list-style-type: none"> <li>Add 0.85% saline to <b>tube #1</b> fill line.</li> <li>Place cap on tube and mix.</li> <li>Centrifuge tube for 60 seconds at 3400-3600 RPMs.</li> <li>Remove saline with a clean transfer pipet.</li> </ol> <p>4.2 Wash with Working Wash Solution *</p> <ol style="list-style-type: none"> <li>Fill tube with working wash to fill line.</li> <li>Place new cap on tube after each wash.</li> <li>Mix gently.</li> <li>Centrifuge tube for 60 seconds at 3400-3600 RPMs.</li> <li>Remove wash solution with a clean transfer pipet.</li> </ol> <p>4.3 Repeat step 4.2 three times for a total of 4 washes.</p> <ol style="list-style-type: none"> <li>Use a transfer pipet to save an aliquot of the supernatant at the red cell line from the last wash to <b>tube # 5</b> to serve as a control.</li> <li>Discarding the last wash requires the eluate to be repeated.</li> <li>Inadequate washing could lead to plasma antibody contamination.</li> </ol> <p><i>* NOTE: Use of the low-ionic strength wash solution provided in the EluKit 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 an eluate that is falsely positive. This is suspected when a known antibody is detected in the eluate and patient has been receiving antigen negative units. Consult with management. A new eluate may need to be prepared using only isotonic saline as the wash agent.</i></p>	

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
5.0	<p><b>Preparing Eluate</b></p> <p>5.1 Transfer 1ml (approximately 20 large drops) of washed red cells to <b>tube # 2</b> using a clean transfer pipet.</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="border: 1px solid black; padding: 2px;">Vertical</div>  <div style="border: 1px solid black; padding: 2px;">Horizontal</div> </div> <p>5.2 Add 20 drops (1ml) of Eluting Solution to elute the antibody.</p> <ol style="list-style-type: none"> <li>a. Maintain a 1:1 dilution by using equal drops of eluting solution and blood if there is less than 1ml.</li> <li>b. Eg. 10 drops of washed cells and 10 drops of eluting solution.</li> </ol> <p>5.3 Cover with plastic cap.</p> <p>5.4 Mix gently by inverting 4-5 times</p> <p>5.5 Centrifuge immediately for 45-60 seconds at 3400-3600 RPMs.</p> <p>5.6 Excess mixing or failure to centrifuge immediately will cause hemolysis which alters the pH of the eluate.</p> <p>5.7 Transfer the supernatant to <b>tube #3</b> using a clean transfer pipet. Discard cells into the red biohazard trash can.</p>	
6.0	<p><b>Buffering Eluate to a neutral pH of 7.0 ± 0.2.</b></p> <p>6.1 Add 5 drops of Buffering Solution to buffer the eluate. Mix well by gently shaking the tube.</p> <p>6.2. Continue adding Buffering Solution dropwise, mixing well after each drop until a blue color appears and remains.(to indicate a pH range of 6.5-7.5)</p> <div style="text-align: center;">  </div> <p>6.3 Check Eluate with pH paper.</p> <ol style="list-style-type: none"> <li>a. Perform QC of pH paper if not done within past week. <i>Refer to Section V: QC of pH paper</i></li> <li>b. Remove one pH strip from container.</li> <li>c. Place pH strip on clean orange biohazard wipe.</li> <li>d. Using a clean transfer pipet, place one drop of eluate on the end of the ph strip.</li> </ol>	

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
	<p>6.4 Compare pH paper with color chart on bottle.</p>  <p>6.5 If not within acceptable pH range, continue adding one drop of buffering solution at a time until the pH is <math>7.0 \pm 0.2</math></p> <p>6.6 Centrifuge the eluate again for 5 minutes to remove cellular debris.</p> <p>6.7 Transfer to a <b>tube # 4</b>. The eluate is ready for testing.</p> <ol style="list-style-type: none"> <li>Store eluate at <math>1^{\circ}</math> to <math>10^{\circ}</math> C if there is a delay in testing.</li> <li>Eluate may be tested up to 7 days after preparation providing no turbidity is present.</li> <li>Do not freeze.</li> </ol>	
7.0	<p><b>Proceed to testing eluate and last wash.</b></p> <p>7.1 Refer to SP: <i>Testing the Acid Eluate in Gel and Gel Ficin.</i></p> <p>7.2 Refer to SP: <i>Testing the Acid Eluate in LISS, PEG, Saline and Ficin Mediums</i></p>	



### III. Procedure: Testing the Acid Eluate in Gel and Gel Ficin

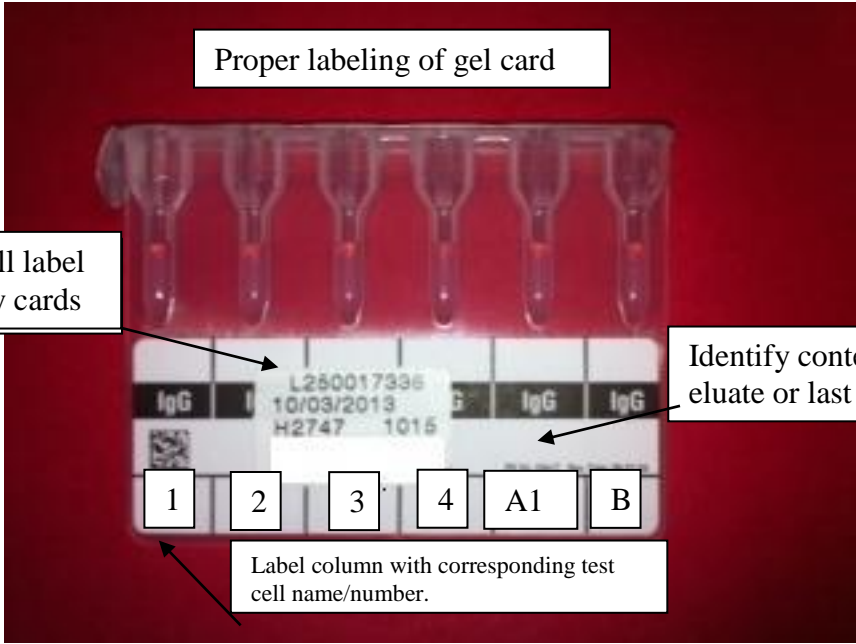
Biological Risk Assessment: Moderate  
 Chemical Risk Assessment None  
 Protective Equipment: Lab Coat/Gloves

**Supplies:** Grifols Pipetor with settings of 12.5ul, 25ul and 50ul ,Pipet Tips, 100µl MLA pipet and tips, Transfer pipets B/B-PET™,12X75 test tubes

**Reagents:** Grifols Anti-IgG Card, Grifols Diluent –store at 2-8° C., 0.8% Screening or Panel cells – store at 2-8° C., 3.0% A1 and B cells – store at 2-8° C.,

**Equipment:** Calibrated Timer, Grifols Centrifuge

**Specimen Requirements:** Pink or lavender top EDTA tube labeled with the patient’s name, medical record number, date of collection and identification of phlebotomist.

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
1.0	<p><b>Determine if antibody screen or panel are needed and label gel cards.</b></p> <p>1.1 For testing, label all Gel cards with small patient label, the number of the cell, and/or the A<sub>1</sub> or B type of the cell.</p> <p style="padding-left: 40px;">a. When a small label is not available handwrite patient name and MRN.</p> <p>1.2 For last wash control, label Gel cards with Screening Cells 1,2,3 in the same manner.</p> <div style="text-align: center;">  </div> <p>1.3 Remove the foil seal from the card leaving the unused wells covered.</p>	



STEPS	INSTRUCTIONS	CHANGE/ APPROVAL															
2.0	<p><b>Dilute A<sub>1</sub> and B cells to 0.8%. Refer to Antibody Identification Techniques, Preparation of 0.8% Cells for Gel testing (if 0.8% are not available).</b></p> <p>2.1 Label 2 12X75 test tubes with Lot # and either A1cells or B cells            2.2 Check that the Grifols pipet is set to 50µl or use the 100µl MLA pipet.            2.3 Using a clean pipet tip, pipet 100µl of Grifols Diluent to each tube.            2.4 Using a clean pipet tip for each cell, pipet 100µl of cells (2-4%) to appropriately labeled tube.            2.5 Centrifuge for 60 seconds at 3400-3600 RPMs in a centrifuge.            2.6 Use a disposable pipet to remove supernatant and discard.            2.7 Using a clean pipet tip, add 200µl of Grifols Diluent to packed red cells and mix.            2.8 Cap the 0.8% A1 cells and B cells and store in reagent refrigerator.            2.9 Expiration time for the diluted A1cells and B cells is 24 hours.            2.10 Label vials with: name of reagent, lot #, revised expiration date and time and prepared by (tech initials) .</p>																
3.0	<p><b>Pipet cells and eluate or last wash into the corresponding gel well.</b></p> <p>3.1 Check that pipet is set to 50ul.            3.2 Using a clean pipet tip for each cell, pipet 50ul of 0.8% screening cells or panel cells and the 0.8% A<sub>1</sub> and B cells            3.3 Change pipet setting to 25ul.            3.4 Using a clean pipet tip, pipet 25uL of eluate to each microtubes labeled eluate            3.5 Using a clean pipet tip, pipet 25uL of Last Wash to microtubes labeled Last Wash.</p> <table border="1" data-bbox="251 1365 1136 1585"> <thead> <tr> <th></th> <th style="text-align: center;">Eluate Cards</th> <th style="text-align: center;">Last Wash Cards</th> </tr> </thead> <tbody> <tr> <td>Screening Cells or Panel</td> <td style="text-align: center;">50ul</td> <td style="text-align: center;">50ul</td> </tr> <tr> <td>A1 and B cells</td> <td style="text-align: center;">50ul</td> <td style="text-align: center;">50ul</td> </tr> <tr> <td>Eluate</td> <td style="text-align: center;">25ul</td> <td style="background-color: black;"></td> </tr> <tr> <td>Last Wash (Control)</td> <td style="background-color: black;"></td> <td style="text-align: center;">25ul</td> </tr> </tbody> </table>		Eluate Cards	Last Wash Cards	Screening Cells or Panel	50ul	50ul	A1 and B cells	50ul	50ul	Eluate	25ul		Last Wash (Control)		25ul	
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STEPS	INSTRUCTIONS	CHANGE/ APPROVAL										
	<p>3.6 When testing CORD eluates:</p> <table border="1" data-bbox="251 333 1146 510"> <thead> <tr> <th>ABO of Baby</th> <th>Cells to test</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>3 A1 cells* and O cells</td> </tr> <tr> <td>B</td> <td>3 B cells* and O cells</td> </tr> <tr> <td>O</td> <td>O cells</td> </tr> <tr> <td>AB</td> <td>3 A1 cells*, 3 B cells* and O cells</td> </tr> </tbody> </table> <p>* 3 A1 and B cells must be from different lots.</p>	ABO of Baby	Cells to test	A	3 A1 cells* and O cells	B	3 B cells* and O cells	O	O cells	AB	3 A1 cells*, 3 B cells* and O cells	
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4.0	<p><b>Incubate at 37° ± 1.5 C (35.5° to 38.5°C) for the appropriate time depending on the strength of the DAT according to the table below.</b></p> <table border="1" data-bbox="399 680 1008 829"> <thead> <tr> <th>DAT Strength</th> <th>Incubation Time</th> </tr> </thead> <tbody> <tr> <td>Wk+ to 1+</td> <td>40 minutes</td> </tr> <tr> <td>2+ or greater</td> <td>15 minutes</td> </tr> </tbody> </table>	DAT Strength	Incubation Time	Wk+ to 1+	40 minutes	2+ or greater	15 minutes					
DAT Strength	Incubation Time											
Wk+ to 1+	40 minutes											
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5.0	<p><b>Centrifuge the gel card in the Grifols Centrifuge at the preset conditions (9 minutes at 870-920 RPM).</b></p>											
6.0	<p><b>Read the front of each microtube macroscopically.</b></p> <p>6.1 Grade and record reactions.</p>											
7.0	<p><b>Interpret results according to table below:</b></p> <table border="1" data-bbox="323 1262 1227 1560"> <thead> <tr> <th>Result</th> <th>Step</th> </tr> </thead> <tbody> <tr> <td><b>Agglutination in Control tubes</b></td> <td><b><u>Repeat Eluate</u></b> Wash quickly and thoroughly</td> </tr> <tr> <td>Agglutination in Eluate and <b>No agglutination in Control</b></td> <td>Identify Antibody Refer to Antibody identification procedure</td> </tr> <tr> <td>No Agglutination in Eluate and <b>Control</b></td> <td>No Antibody Recovered</td> </tr> </tbody> </table>	Result	Step	<b>Agglutination in Control tubes</b>	<b><u>Repeat Eluate</u></b> Wash quickly and thoroughly	Agglutination in Eluate and <b>No agglutination in Control</b>	Identify Antibody Refer to Antibody identification procedure	No Agglutination in Eluate and <b>Control</b>	No Antibody Recovered			
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No Agglutination in Eluate and <b>Control</b>	No Antibody Recovered											
8.0	<p><b>Record results on appropriate antibody identification forms.</b></p>											
9.0	<p><b>Discard gel cards in Red Biohazard trash cans.</b></p>											

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL								
10.0	<p><b>Enter eluate interpretation in SCC.</b></p> <p>10.1 Order elution in SCC.</p> <p>a. ELU – To result up to 4 antibodies</p> <p>b. ELU2 – If 5 to 8 antibodies</p> <p>Result eluate as follows:</p> <table border="1" data-bbox="358 541 1170 877"> <thead> <tr> <th data-bbox="358 541 561 583">Reactions</th> <th data-bbox="561 541 1170 583">Enter in SCC for eluate</th> </tr> </thead> <tbody> <tr> <td data-bbox="358 583 561 657">All cells are nonreactive</td> <td data-bbox="561 583 1170 657">Result: Negative</td> </tr> <tr> <td data-bbox="358 657 561 768">All cells are reactive</td> <td data-bbox="561 657 1170 768">Result: Positive AND ALL (All cells are positive) or WARM (warm autoantibody)</td> </tr> <tr> <td data-bbox="358 768 561 877">Specific antibody</td> <td data-bbox="561 768 1170 877">Result: Positive AND Result antibody identified in eluate e.g. Anti-Fya would be ANFYA</td> </tr> </tbody> </table> <p>10.2 Review results with management when it does not agree with clinical post or present patient findings.</p>	Reactions	Enter in SCC for eluate	All cells are nonreactive	Result: Negative	All cells are reactive	Result: Positive AND ALL (All cells are positive) or WARM (warm autoantibody)	Specific antibody	Result: Positive AND Result antibody identified in eluate e.g. Anti-Fya would be ANFYA	
Reactions	Enter in SCC for eluate									
All cells are nonreactive	Result: Negative									
All cells are reactive	Result: Positive AND ALL (All cells are positive) or WARM (warm autoantibody)									
Specific antibody	Result: Positive AND Result antibody identified in eluate e.g. Anti-Fya would be ANFYA									

**IV. Testing the Acid Elution in LISS, PEG, Saline and Ficin mediums.**

Biological Risk Assessment: Moderate  
 Chemical Risk Assessment: None  
 Protective Equipment: Lab Coat/ Gloves

**Supplies:** Transfer pipets B/B-PET™ , 12X75 test tubes, Biohazard wipes

**Reagents:** 0.85% Certified Blood Bank Saline for cell suspensions, 0.85% Certified Blood Bank Phosphate Buffered Saline for cell washers, LISS- store at 2-8° C., PEG- store at 2-8° C., Anti-IgG- store at 2-8° C., IgG sensitized cells- store at 2-8° C., Working Wash Solution- store at 2-8° C.

**Equipment:** Calibrated Timer, Serofuge 3400-3600 RPM, Cellwasher 3400-3600 RPM

**Specimen Requirements:** Pink or lavender top EDTA tube labeled with the patient’s name, medical record number, date of collection and identification of phlebotomist.

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL						
1.0	<p><b>Determine if antibody screen or panel are needed and label tubes.</b></p> <p>1.1 For testing, label all 10X75 tubes with the first 3 initials of the patient’s last name, the number of the cell, and/or the A<sub>1</sub> or B type of the cell.</p> <p>1.2 For last wash control, label 10X75 tubes Screening Cells 1, 2, 3 and A<sub>1</sub> and B cells in the same manner.</p>							
2.0	<p><b>Prepare a dry cell button of cells being tested.</b></p> <p>2.1 Add one drop of 3-5% panel/screen/A or B cells into each labeled tube.</p> <p>2.2 Add one (1) drop of 0.85% saline to each tube cell to be tested.</p> <p>2.3 Centrifuge for 20 seconds 3400-3600 RPM.</p> <p>2.4 Decant by quickly turning the tube upside down and allowing the saline to flow to the mouth of the tube where it can be removed with a biohazard wipe leaving the cell button behind and intact.</p>							
3.0	<p><b>Review the strength of the DAT to determine the amount of eluate to add to the corresponding dry cell button in tube.</b></p> <table border="1" data-bbox="355 1570 1159 1692"> <thead> <tr> <th data-bbox="355 1570 773 1612">DAT Strength</th> <th data-bbox="773 1570 1159 1612">Amount of Eluate to add</th> </tr> </thead> <tbody> <tr> <td data-bbox="355 1612 773 1654">Wk + to 1+</td> <td data-bbox="773 1612 1159 1654">4 drops</td> </tr> <tr> <td data-bbox="355 1654 773 1692">2+ or greater</td> <td data-bbox="773 1654 1159 1692">2 drops</td> </tr> </tbody> </table> <p>3.1 PEG is the potentiator of choice. Consult with management if questions.</p>	DAT Strength	Amount of Eluate to add	Wk + to 1+	4 drops	2+ or greater	2 drops	
DAT Strength	Amount of Eluate to add							
Wk + to 1+	4 drops							
2+ or greater	2 drops							

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL															
4.0	<p><b>Incubate at 37° ± 2 C (35° to 39°C).</b></p> <p>4.1 If using LISS, PEG or Ficin, incubate a minimum of 15 minutes to a maximum of 30 minutes depending on the strength of the DAT.</p> <table border="1" data-bbox="399 457 1008 604"> <thead> <tr> <th>DAT Strength</th> <th>Incubation Time</th> </tr> </thead> <tbody> <tr> <td>Wk+ to 1+</td> <td>30 minutes</td> </tr> <tr> <td>2+ or greater</td> <td>15 minutes</td> </tr> </tbody> </table> <p>4.2 If using Saline incubate for 60 minutes. a. Refer to: SP: Antibody Identification</p>	DAT Strength	Incubation Time	Wk+ to 1+	30 minutes	2+ or greater	15 minutes										
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Wk+ to 1+	30 minutes																
2+ or greater	15 minutes																
5.0	<p><b>Remove tubes from 37°C incubator.</b></p> <p>5.1 Using LISS,PEG ,Saline or Ficin see the following chart:</p> <table border="1" data-bbox="253 947 1308 1524"> <thead> <tr> <th>Medium</th> <th>After 37° incubation</th> <th>Wash in cell washer</th> </tr> </thead> <tbody> <tr> <td><b>LISS</b></td> <td><b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently.</b></td> <td><b>NA</b></td> </tr> <tr> <td><b>PEG</b></td> <td><b>Check for hemolysis DO NOT SPIN</b></td> <td><b>4 times</b></td> </tr> <tr> <td><b>Saline</b></td> <td><b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently</b></td> <td><b>NA</b></td> </tr> <tr> <td><b>Ficin</b></td> <td><b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently.</b></td> <td><b>NA</b></td> </tr> </tbody> </table> <p>5.2 Add 10 drops of the <b>1°C-10°C</b> Working Wash Solution to the tubes and mix. a. NA for PEG – should be washed four (4) times in cell washer.</p> <p>5.3 Centrifuge for 30 seconds at 3400-3600 RPM unless using PEG.</p> <p>5.4 Decant and blot dry. (see Step 2.3 of this procedure)</p> <p>5.5 Refer to Cell Washer Operating Procedure <a href="#">BB.EQUIP.1027</a></p>	Medium	After 37° incubation	Wash in cell washer	<b>LISS</b>	<b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently.</b>	<b>NA</b>	<b>PEG</b>	<b>Check for hemolysis DO NOT SPIN</b>	<b>4 times</b>	<b>Saline</b>	<b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently</b>	<b>NA</b>	<b>Ficin</b>	<b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently.</b>	<b>NA</b>	
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<b>Saline</b>	<b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently</b>	<b>NA</b>															
<b>Ficin</b>	<b>Spin for 20 seconds at 3400-3600 RPM. Check for hemolysis. Resuspend gently.</b>	<b>NA</b>															

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL								
6.0	<p><b>Add 2 drops of Anti IgG</b></p> <p>6.1 Mix well 6.2 Centrifuge for 20 seconds at 3400-3600 RPMs. Note: With Management direction, Poly DAT and C3d/C3b complement Coombs may need to be substituted in certain patient workups.</p>									
7.0	<p><b>Resuspend and read macroscopically and microscopically for agglutination.</b></p>									
8.0	<p><b>Grade and record test results immediately.</b></p>									
9.0	<p><b>Confirm all negative reactions by adding IgG sensitized cells</b></p> <p>9.1 Mix and centrifuge for 20 seconds at 3400-3600 RPMs. 9.2 Repeat the test if there is no agglutination.</p>									
10.0	<p><b>Interpret results according to the following table:</b></p> <p style="text-align: center;"><b>Interpretation of Eluate and Control</b></p> <table border="1" data-bbox="323 1081 1227 1381"> <thead> <tr> <th data-bbox="323 1081 776 1121">Result</th> <th data-bbox="776 1081 1227 1121">Step</th> </tr> </thead> <tbody> <tr> <td data-bbox="323 1121 776 1194">Agglutination in Last Wash (Control) tubes</td> <td data-bbox="776 1121 1227 1194">Repeat Eluate Wash quickly and thoroughly</td> </tr> <tr> <td data-bbox="323 1194 776 1308">Agglutination in Eluate and No agglutination in Last Wash tubes</td> <td data-bbox="776 1194 1227 1308">Identify Antibody Refer to Antibody identification procedure BB.SP.1002</td> </tr> <tr> <td data-bbox="323 1308 776 1381">No Agglutination in Eluate or Last Wash tubes</td> <td data-bbox="776 1308 1227 1381">No Antibody Recovered</td> </tr> </tbody> </table>	Result	Step	Agglutination in Last Wash (Control) tubes	Repeat Eluate Wash quickly and thoroughly	Agglutination in Eluate and No agglutination in Last Wash tubes	Identify Antibody Refer to Antibody identification procedure BB.SP.1002	No Agglutination in Eluate or Last Wash tubes	No Antibody Recovered	
Result	Step									
Agglutination in Last Wash (Control) tubes	Repeat Eluate Wash quickly and thoroughly									
Agglutination in Eluate and No agglutination in Last Wash tubes	Identify Antibody Refer to Antibody identification procedure BB.SP.1002									
No Agglutination in Eluate or Last Wash tubes	No Antibody Recovered									
11.0	<p><b>Record results on appropriate antibody identification forms.</b></p>									
12.0	<p><b>Discard test tubes in Red Biohazard trash cans.</b></p>									
13.0	<p><b>Record in SCC:</b></p> <p>13.1 Review results with management when it doesn't agree with clinical past or present patient findings. <i>Refer to Section III, Step 10.</i></p>									

## V. QC of pH Paper

Biological Risk Assessment: Moderate  
 Chemical Risk Assessment: None  
 Protective Equipment: Lab Coat/ Gloves

**Supplies:** pH paper strips  
**Reagents:** Level 1 and Level 2 dipstick controls  
**Equipment:** NA  
**Specimen Requirements:** NA

STEPS	INSTRUCTIONS	CHANGE/ APPROVAL
1.0	<b>Remove 2 pH strips from container.</b>	
2.0	<b>Add 1 drop of Level 1 to one strip.</b>	
3.0	<b>Add 1 drop of Level 2 to the other strip.</b>	
4.0	<b>Record pH results on Rare Antisera QC sheet.</b>	
5.0	<b>Check dipstick control insert for expected range.</b>	
6.0	<b>Notify management if controls do not work.</b>	

### 3. Review/Revised/implemented:

All procedures must be reviewed according to Document Control Protocol.  
 All new procedures and procedures that have major revisions must be signed by the CLIA Director.  
 All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director or designee.

### 4. Related Procedures:

*Special Procedures: Antibody Identification Protocol*

### 5. References: Technical Manual American Association of Blood Bank, Revised Periodically Gamma Elu-Kit<sup>tm</sup> II package insert Immucor, Revised Periodically Ortho MTS Procedure Manual; Revised Periodically

### 6. Attachments: NA

### 7. Revised/Reviewed Dates and Signatures:

See Document Change Control

Document Change Control										
Title: Acid Elution										
Previous title:										
Written date				Written by:						
Validation date				Validation by						
Reviewed date				Reviewed by						
Approved date				Approved by						
Approved date				Approved by						
Effective date in use		<7/2009		In use by		See Archive Record				
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By	
12/11/12	KJ	GP	4/15/14	4/10/14	EF	4/10/14	JHS	4/15/14	JHS	
Validate Date	By	Revisions: Working Wash solution utilizing entire amount in bottle. Making an eluate from an amount of RBC's less than 1ml added. Utilization of pH strips for proper buffer range added.								
04/03/14	LW									
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By	
7/1/14	KJ			7/28/14	EF	7/16/14	MRJ	7/30/14	JHS	
Validate Date	By	Revisions: Added QC of pH paper to procedure. In Section IV, step 5 added NA to wash in cell washer for LISS, Saline and Ficin.								
7/3/14	JHS									
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By	
4/5/16	JJ	6/14/16	GP	6/10/16	EF	6/10/16	MRJ	6/14/16	JHS	
Validate Date	By	Revisions: Changed procedure for making 0.8% cell suspension from 2-4% cell suspension in section III step 2.0.								
6/8/16	NJ	Added Ortho MTS Procedure manual to references.								
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By	
2/20/19	JHS			2/20/19	EF	2/20/19	JHS	2/21/19	JHS	
Validate Date	By	Revisions: Removed Sunquest and added SCC. Removed references to Ortho and replaced with Grifols. Added SCC to definitions. Added * NOTE: Use of the low-ionic strength wash solution provided in the EluKit 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 an eluate that is falsely positive. This is suspected when a known antibody is detected in the eluate and patient has been receiving antigen negative units. Consult with management. A new eluate may need to be prepared using only isotonic saline as the wash agent.								
2/20/19	LA									
Locations				Out of Use: Date:		By				
				Reason						

Reviews: Record date/initials

Date	Initials	Date	Initials	Date	Initials	Date	Initials
10/21/09	GP	10/27/09	MRJ	11/17/10	GP	05/14/13	EF
09/05/13	GP	10/01/13	MRJ	11/9/15	JHS	11/21/17	JHS
2/20/19	JHS						



Document Change Control									
Title: Acid Elution									
Previous title:									
Written date		Written by:							
Validation date		Validation by							
Reviewed date		Reviewed by							
Approved date		Approved by							
Approved date		Approved by							
Effective date in use	<7/2009	In use by						See Archive Record	
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By
2/25/20	CSW								
Validate Date	By	Revisions: Added selected cells table for running Cord blood eluates. Removed Mary Rose Jones.							
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By
Validate Date	By								
Revised Date	By	MD Date	By	MD Date	By	Review Date	By	Effective Date	By
Validate Date	By								
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Validate Date	By								
		Out of Use Date:		By:					
		Reason							