Exercise 9  Elution Study

Objectives:
1. State when an elution procedure may be performed.
2. List four situations in which the performance of an elution may provide helpful information.
3. List three types of elutions procedures which may be performed to recover IgG antibodies coating red blood cells.
4. State the advantages of the Lui freeze-thaw elution over other types of elution procedures.
5. State the principle of the elution procedure.
6. State the purpose of the last wash control.
7. State the cause of positive reactions in the last wash control.
8. State the reason that an eluate performed on a cord blood from a group O mother will react with the reverse cells which are not the same type, i.e., baby is group A but reactions are obtained with the B cells.
9. Given the reactions of a cord blood work up interpret the results.

Introduction

The direct antiglobulin test (DAT) is used to detect immunoglobulin G (IgG) molecules or complement fragments (C3d) on the surface of red blood cells. When the DAT is positive due to coating with IgG it is important to determine the specificity of the antibody by performing an elution. When the DAT is positive with anti-IgG an elution procedure may be performed. Elutions are performed to remove antibody from IgG coated red blood cells. The eluate is then tested against reagent red blood cells to determine the specificity. Performing an elution may provide helpful information in the following situations:

1. To investigate a suspected delayed transfusion reaction.
2. To investigate fetal-maternal incompatibility.
3. To investigate drug-induced phenomena.
4. To resolve multiple antibody specificities present in a single serum/plasma.

There are many different types of elution procedures which are used to recover IgG antibodies from coated red cells: Lui freeze-thaw, heat, digitonin-acid, ether, and chloroform, just to name a few. The elution procedure selected will be determined by the situation being evaluated. For example, in a delayed transfusion reaction (DTR) the DAT on the patient is positive. The antibody screen may be positive or negative if all antibody produced is coating the donor red blood cells in the circulation. Since an immune antibody is suspected a heat, digitonin-acid or chloroform eluate may be performed. In a DTR the patient antibody is removed from antigen positive donor red cell. The eluate is then tested against reagent red cells to determine the specificity.
When ABO antibody coating of red blood cells only is suspected a Lui freeze-thaw elution is the best choice. Other elutions could be performed but the Lui freeze-thaw has the advantage of not requiring any special elution reagents and is easy to perform. The Lui freeze may be used when a patient has a positive DAT, negative screen and has only been transfused with ABO incompatible platelets. The most common reason for performing the Lui freeze-thaw is in the investigation of a positive DAT on a cord blood due to ABO incompatibility. In this case the mother is group O, the antibody screen is negative, the baby is an A or B and the DAT is positive. The antibody recovered is tested against A, B and screen cells. The expectation is that the A or B cells will be positive and the screen cells will be negative since ABO antibodies are expected to be recovered.

**Principle**

In an elution procedure, the cells are washed free of all unbound antibody and a procedure is performed on the cells to cause the release of the antibody molecules from the cells into a solution termed the “eluate”. The eluate contains the antibodies which were removed from the red blood cells and is then tested against reagent red cells to determine the specificity. Remember that any antibody recovered in the eluate prepared from the cord cells is of maternal origin.

The most critical part of the procedure is thorough washing. Washing is performed to remove all unbound antibody from the test system. Failure to wash adequately will result in unbound antibody being present in the eluate. An aliquot of saline is saved from the last wash and serves as a negative control for the procedure. If the last wash is positive this indicates the presence of unbound antibody and invalidates the test and the test must be repeated on a more thoroughly washed red cell sample. It is crucial to thoroughly resuspend the red blood cells between each wash as unbound antibody will be trapped and will contaminate the eluate. This is done by filling the tube with saline and with the cap in place inverting the tube until there are no red cells clumped at the bottom of the tube. Thumping the tube with your finger when the tube is inverted will assist in resuspending the red cells.
Lui Freeze-Thaw Procedure

Sample Required
1. EDTA red blood cells are preferred.
2. Red blood cells from clotted specimen.

Reagents and Supplies
1. Test tubes 12x75
2. Disposable pipettes
3. Normal saline
4. A1 and B cells
5. Screen cells
6. Anti-IgG
7. Check Cells

Procedure
1. Label a tube with the mother’s name and hospital number and “cord cells”.
2. Label two additional tubes with the mother’s first and last initials and one with “eluate” and the other with “last wash control”.
3. Transfer 20 drops of red packed red blood cells into the tube labeled with the mother’s name and number.
4. Wash the 20 drops of red cells 6 times with normal saline. IMPORTANT: Use a pipette to remove the saline from the tube. Be careful to not remove any red cells.
5. On the 6th wash remove the saline and place into the tube labeled “last wash”.
6. To the 20 drops of washed red blood cells add three drops of saline.
7. Place a purple cap on the tube OR cover tightly with parafilm.
8. Rotate the tube in order to coat the inside of the tube with red cells.
9. Place the tube on its side in a freezer (-20C to -70C) for 10 minutes or until frozen.
10. While the red cells are freezing label 2 sets of 5 tubes as follows:
    a. Write “EL”, for eluate, at the top of the tube and AC, BC, S1, S2 and S3.
    b. Write “LW”, for last wash, at the top of the tube and AC, BC, S1, S2 and S3.
11. Thaw the red cells rapidly under warm running tap water.
12. Centrifuge the thawed cell hemolysate/eluate in the serofuge for 5 minutes.
13. Carefully, using a light source, transfer the eluate to the tube labeled “eluate”.
14. To the set of 5 tubes labeled “EL” add 2 -3 drops of the eluate. HINT: Add 2 drops to each of the 5 tubes, if there is enough eluate left add one additional drop. The eluate contains antibodies recovered from the cells, the more eluate added to the test, the more antibody available to attach to antigens on cells.
15. To the set of 5 tubes labeled “LW” add the same number of drops of last wash as the number of drops of eluate. For example, if you added 2 drops of eluate to each tube, add 2 drops of last wash to the LW tubes.
16. Add 1 drop of the appropriate reagent cell to each tube.
17. Mix thoroughly and incubate at 37°C for 30 minutes.
18. After incubation wash 3 times with saline, blot after the last wash.
19. Add 2 drops of anti-IgG to each tube, mix thoroughly.
20. Centrifuge for 15 seconds.
21. Read tubes macroscopically. Read all negative tubes microscopically.
22. Record results with tube held in your hand.
23. See “Interpretation” to interpret the results.

**Interpretation**

1. **If the last wash is positive with any of the test cells, the original cell sample was probably under washed and the elution procedure should be repeated on a more thoroughly washed cell sample.**
2. Lack of agglutination of test cells by the eluate indicates absence of antibodies specific for the antigens on the test cells and the test is reported out as “Negative”.
3. If “last wash” tubes are negative, any agglutination of reagent cells with eluate indicates recovery of antibodies from the original cells. In the case of cord blood, the interpretation should be reported as follows:

<table>
<thead>
<tr>
<th>Reactions with Cells</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 B S1 S2 S3</td>
<td></td>
</tr>
<tr>
<td>+ O O O O</td>
<td>Immune anti-A eluted</td>
</tr>
<tr>
<td>O + O O O</td>
<td>Immune anti-B eluted</td>
</tr>
<tr>
<td>+s +w O O O</td>
<td>Immune anti-A and anti-A,B eluted</td>
</tr>
<tr>
<td>+w +s O O O</td>
<td>Immune anti-B and anti-A,B eluted</td>
</tr>
<tr>
<td>O O (+ on any cell)</td>
<td>Unexpected antibody. Perform panel on maternal serum</td>
</tr>
</tbody>
</table>

If the mother is group O and the baby is group A or B, it is not unusual to obtain positive reactions with both the A and B cells. This reactivity is due to the anti-A,B present in the group O mother’s serum which has coated the baby’s red blood cells. This antibody will react with both A and B cells.

Use the interpretation chart above along with your common sense to interpret your data. If the mom is group O and the baby is group A, it is impossible to elute an immune anti-B from the baby cells. Think your interpretation through carefully.
For the example below the mom is group O, antibody screen negative and the baby is group A with a positive DAT. The following elution results were obtained.

<table>
<thead>
<tr>
<th></th>
<th>A&lt;sup&gt;1&lt;/sup&gt; cells</th>
<th>B cells</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate</td>
<td>4+</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Last Wash Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Interpretation: Immune anti-A and -A,B eluted from cord cells.
Exercise 9: Elution Studies
Recording Results

Mother's Name ________________________________
Hospital Number ________________________________
Baby's DAT ________________________________
Baby's ABO/D Typing ________________________________
Mother's ABO/D Typing ________________________________
Mother's Antibody Screen ________________________________

<table>
<thead>
<tr>
<th>Cells</th>
<th>Reactions with Eluate</th>
<th>Reactions with Last Wash Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A^1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation of elution results: ________________________________
Exercise 9  Elution Procedure  
Study Questions

1. What is the *principle* of the elution procedure? (1 point)

2. List four (4) pieces of information that are useful in the workup of a positive DAT. (2 points)

3. What determines which type of elution procedure to use for an HDN workup? (1 point)

4. What type of elution is used for ABO-HDN? (1 point)

5. In the elution procedure, why must the cells be thoroughly washed? (1 point)

6. What is the most probable cause of positive results with the last wash control? (1 point)
7. What must be done if the last wash control gives positive reactions? (1 point)

8. What does lack of agglutination of the test cells by the eluate indicate? (1 point)

9. Write your interpretation of the following results obtained in an elution procedure in the space provided below. (1 point)

**Baby is A positive**
**Mom is 0 positive, screens are negative**

<table>
<thead>
<tr>
<th></th>
<th>A1 cells</th>
<th>B cells</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate</td>
<td>4+</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Last Wash</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Interpretation:**

10. When the mother is group O and the baby is A or B why is it common to get reactions in the eluate with both the A and B cells? (1 point)