Exercise 10  

Rh Immune Globulin Workup (RhIgW)

Task
To perform one (1) Rh immune globulin workup.

Aim
With 100% accuracy, determine whether or not Rh immune globulin is indicated and how much by performing the appropriate tests.

Introduction
Rh immune globulin (RhIg) is used to prevent immunization of D negative individuals who have been exposed to the D antigen.

One group at increased risk of exposure and sensitization by the D antigen are D negative mothers who give birth to D positive infants. During delivery of the baby, Rh positive fetal cells enter the maternal circulation when the placenta separates from the uterine wall. In general, only a small amount of fetal blood (less than 30 ml whole blood) enters the mothers circulation, but due to the immunogenicity of the D antigen, this is enough to cause sensitization. If a sensitized woman becomes pregnant with another D positive baby, the baby will be affected with severe HDN.

HDN due to anti-D is the most severe type. The bilirubin level rises quickly after birth. If it exceeds 20 mg/dL kernicterus may occur. Kernicterus is caused when the basal ganglia and other areas of the brain and spinal cord are infiltrated with bilirubin resulting in mental retardation or death if not treated. In addition to the high bilirubin levels, these infants become severely anemic due to the destruction of their red blood cells by the maternal antibody. To prevent this sensitization to the D antigen, Rh Immune Globulin (RhIg) was developed and became available in the early 1960s. This product contains anti-D which, when injected intramuscularly within 72 hours following delivery of an Rh positive baby, will attach to the D antigen on the baby's RBCs. This would result in the baby's cells being recognized as foreign and removed from the mother's circulation before her immune system could recognize the D antigen as foreign, thus preventing her from becoming immunized. One vial of RhIg contains 300 mg of anti-D, enough to cover a 30 ml whole blood bleed or 15 mls of packed cells.

Later, it was discovered that even though the D negative mothers were identified and given RhIg within the appropriate time frame, there was still a 1% failure rate. That is to say, one per cent of the mothers who received RhIg after delivery were subsequently discovered to be immunized to the D antigen. It was then determined that very small feto-maternal hemorrhages were occurring during the third trimester. It is now routine practice for physicians to administer a dose of RhIg at 28 weeks gestation, in addition to the post partum dose. Ante partum administration of RhIg has decreased the overall risk of immunization to 0.1%. No adverse effects have been observed in infants of mothers who have received up to two doses of antenatal RhIg although it may cause the baby to have a positive DAT.
After delivery of an infant, certain tests are performed to determine whether or not a woman is to be considered an RhIg candidate. The following women are not candidates for RhIg:

1. D negative women who have D negative babies
2. D positive women
3. D negative women known to be immunized to D.

RhIg must be given to D negative women under the following circumstances in which the baby's D is unknown:

1. after amniocentesis
2. after miscarriage
3. after abortion
4. after ectopic pregnancy
5. vaginal bleeding at anytime during the pregnancy
6. cordocentesis
7. chorionic villus sampling

RhIg must also be given if the D type of the infant cannot be determined after birth as may be due to the infant having a positive DAT. In these situations the D and/or Rh control tubes are positive at the AHG phase during D\(^a\) testing. This is the reason that a D control or DAT must be performed on any individual who appears weak D positive. Unless the mother is D positive, the best rule of thumb to follow is when in doubt, give it.

**Principle**

After an D negative woman gives birth to an infant, a type and D is ordered on the infant's cordblood. A DAT may also be ordered. If the baby is D positive, an Rh immune globulin workup is ordered on the mother. This includes:

1. ABO/D typing, including micro D\(^a\)
2. Antibody screen
3. Rosette procedure

Each of the above tests serves a specific purpose:

1. It is very important to accurately determine the D type of the mother. If a mother turns out to be S positive, or weak D positive, she is not an RhIg candidate. The D\(^a\) must be read microscopically. This is one way in which an excessive feto-maternal hemorrhage may be detected. If excessive D positive fetal cells are in the maternal circulation they will be coated by the anti-D and form clumps when the AHG serum is added. A “mixed-field” appearance in the anti-D tube and a negative Rh control is the first indication that an excessive bleed has occurred.

2. An antibody screen is routinely performed to identify those women who have formed or are forming an immune anti-D. Occasionally a mother's screens may
be positive due to the ante partum administration of the RhIg. In these cases the antibody screen is very, very weak and once identified as being due to RhIg administration, she must still get a post partum dose. If it has been determined that the anti-D is immune, she is not an RhIg candidate. It is critical that her history be obtained, specifically when her dose of ante partum RhIg was administered. The term used for the anti-D detected after administration of RhIg is “passively acquired anti-D”.

3. The third part of the Rh immune globulin workup is the erythrocyte rosetting test. The brand name of the kit used in lab is FETALSCREEN. This test is performed to screen for an abnormally large feto-maternal hemorrhage which would indicate the need for more than 1 vial of RhIg.

**Principle of the Rosette Test**

A suspension of red cells from the D negative mother is mixed with FETALSCREEN Antibody Reagent (anti-D), incubated, and washed to remove unbound antibody. During the incubation period, any D positive fetal red cells present will become sensitized with anti-D. One antigen binding site of each antibody molecule (Fab piece) attaches to a fetal cell leaving the other antibody site free to attach to a D antigen of the FETALSCREEN Indicator Cells (D positive cells) which are added subsequently. This suspension of test cells and indicator cells is centrifuged briefly, transferred to a microscope slide, or read the tube on the tube holder and examined for clumps of agglutinated red cells, using low power magnification.

The result is an appearance of rosettes or mixed-field agglutinates in which the agglutinates consist of clumps of D positive indicator cells clustered about the sensitized fetal cells. Each clump seen represents one fetal cell surrounded by the indicator cells. If a clump of cells is seen in the micro D⁰, each cell in the clump is a fetal cell. The rosette test is so sensitive and specific that there must be a certain number of clumps seen before it is called positive, while in the micro D⁰ the presence of even one clump is significant. When the test sample contains few or no D positive fetal cells, rosetting is not observed. Because the vast majority of samples screened will contain very few D positive fetal red cells, observing measurable numbers of rosettes in a test should alert the technologist to the possibility that a larger than expected feto-maternal hemorrhage may have occurred. In this situation, a quantitative test must be used to determine the amount of feto-maternal hemorrhage and the dosage of Rh immune globulin required.

Weak D positive red cells may not react as well as D positive red cells in rosetting procedures, presumably because they have fewer D antigen sites. *If the newborn types as Rh weak D positive, a Kleihauer-Betke acid elution test should be done routinely.*

The Rosette procedure is much more sensitive than the micro D⁰ in detecting excessive feto-maternal hemorrhages.

A positive D⁰ and/or rosette procedure indicates that an excessive feto-maternal hemorrhage has occurred. The D⁰ and rosette are *qualitative* tests. They can determine that an excessive bleed has occurred, but not how much.
The method used to quantitate the bleed is the Kleihauer-Betke acid elution stain. This method is based on the fact that fetal hemoglobin (hemoglobin F) is resistant to acid elution, whereas adult hemoglobin is not. When a thin blood smear is exposed to an acid buffer, the adult red blood cells lose their hemoglobin into the buffer so that only stroma remains. Normal adult cells appear as very pale ghosts. Fetal red cells are unaffected and retain their hemoglobin. Fetal cells appear as bright pink refractile bodies. The volume of fetal cells is determined by recording the number of fetal cells observed in 2000 adult cells and converting it to percentage using the following formula:

$$\frac{\text{number of fetal cells}}{2000} \times 100 = \text{percent fetal cells}$$

Then determine the volume of feto-maternal hemorrhage in ml of whole blood by taking the percent fetal cells x 50.

% fetal cells x 50 = volume of feto-maternal hemorrhage

One vial of RhIg will cover a 30 ml whole blood bleed. If it is determined that the bleed exceeds this amount, the volume of fetal bleed is divided by 30 to determine the number of doses of RhIg required.

$$\frac{\text{volume of hemorrhage}}{30} = \text{number of vials}$$
REAGENTS

1. See page 1
2. Rosette kit (fetal bleed screen)

PROCEDURE

1. Perform DAT on cordbloods as in DAT exercise.
2. Perform ABO/D typing on the cordbloods of all D negative mothers.
3. If mother is D negative and baby is D positive, obtain maternal specimen from instructor for RhIg work up.
4. Place maternal serum in appropriately labeled tube and prepare a 4-6% patient cell suspension (mother's cells).
5. Correctly label tubes for Type and Screen, remember to set up Rh ctrl tube.
6. Label three (3) additional tubes for rosette test as follows: patient initials ROS, “ROS +” (positive control) and “ROS=” (negative control).
7. a. Add reagents serum and cells, and patient serum and cells to the appropriate type and screen tubes.
   b. Add: one drop patient cells to patient ROS tube; one drop of rosette pos control cells to + ROS tube; and one drop of rosette neg control cells to = ROS tube.
8. Add one drop of Fetalscreen Antibody reagent to the three (3) rosette test tubes, mix well and immediately place in 37°C water bath.
9. Spin Type and Screen tubes. Read and record reactions immediately. (Saline IS phase)
10. Add two (2) drops of albumin to the antibody screen. Mix well and place those tubes plus the patient's negative D and Rh ctrl tube in the 37°C for 20 minutes. (If the patient is D positive, see instructor.)
   HELPFUL HINT: At this time, you should have eight tubes incubating at 37°C. If you don't, see instructor.
11. After incubation, spin the three (3) antibody screen tubes for 20 seconds, read and record reactions immediately. (Albumin, 37°C phase)
12. Remove the D tube, Rh ctrl and 3 ROS tubes from waterbath and wash all eight (8) tubes with saline three (3) times, blotting ends of tubes with gauze after last wash.
   Read the following steps CAREFULLY.
13. a. Add two (2) drops of anti-human globulin serum to the screen tubes, anti-D tubes, and Rh ctrl Tube
   b. Add one drop of Fetalscreen Indicator cells to all three (3) rosette tubes.
   c. Add one (1) drop of Fetalscreen Enhancement Reagent to the three (3) rosette tubes.
14. Mix all eight (8) tubes well and spin for 20 seconds.
15. Read all tubes macroscopically and microscopically and record reactions.
16. Use check cells to confirm AHG reagent activity in all appropriate tubes (negative D and screens). DO NOT use check cells in the three rosette tubes.
17. Record results of the fetal bleed screen in the box marked “FBS” on the patient form.

INTERPRETATION OF RESULTS

Antibody screen – Refer to previous procedure.
Rh and Rh Control/Microscopic D*  

<table>
<thead>
<tr>
<th>anti-D</th>
<th>D*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>Rh negative</td>
</tr>
<tr>
<td>*</td>
<td>O 3+</td>
<td>Weak D positive</td>
</tr>
<tr>
<td>O</td>
<td>±MF</td>
<td>possible excessive feto-maternal hemorrhage – perform Kleihauer</td>
</tr>
<tr>
<td>*</td>
<td>O +</td>
<td>test invalid-perform Kleihauer</td>
</tr>
</tbody>
</table>

*Perform DAT on patient. If it is negative, patient is a weak D positive. If it is positive, additional work would need to be done.

Fetal Screen/Rosette Test

<table>
<thead>
<tr>
<th>Patient</th>
<th>Positive Control</th>
<th>Negative Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O/+ (less than 7 clumps in 5 fields)</td>
<td>+</td>
<td>O</td>
<td>excessive bleed has not occurred</td>
</tr>
<tr>
<td>+ (greater than 7 clumps in 5 fields)</td>
<td>+</td>
<td>O</td>
<td>excessive bleed has occurred, perform Kleihauer</td>
</tr>
<tr>
<td>O</td>
<td>+</td>
<td>+</td>
<td>invalid, repeat test</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>invalid, repeat test</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td>invalid, repeat test</td>
</tr>
</tbody>
</table>
## Cordblood Flow Chart
### Direct Antiglobulin Test – DAT

<table>
<thead>
<tr>
<th>DAT Negative</th>
<th>DAT Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mom D pos</td>
<td>ABO/D type cord cells</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mom D Neg</td>
<td>Type and Screen Mom</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Nothing more</td>
<td>Screen Negative and Mom is Group O and baby is A or B</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>ABO/D type cord cells</td>
<td>Screen Positive</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>D neg baby</td>
<td>Panel on mom</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>D pos baby</td>
<td>Lui Freeze elution Identify antibody in mom's serum</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>No RhIg</td>
<td>interpret results</td>
</tr>
<tr>
<td></td>
<td>Acid eluate on cord cells</td>
</tr>
<tr>
<td></td>
<td>Identify antibody in cord eluate</td>
</tr>
<tr>
<td></td>
<td>No RhIg needed for Mom</td>
</tr>
</tbody>
</table>

For D negative mothers in which the baby has a positive DAT, RhIg candidacy is determined by identification of the antibody coating the baby's cells.

- If it is due to *immune* anti-D (determined by mother's history) she is *not* an RhIg candidate.
- If it is due to passively acquired anti-D from her antenatal dose of RhIg (determined by mother's history) she *is* an RhIg candidate.
- If it is due to any other immune antibody (i.e., K, Fy, A etc.) she *is* an RhIg candidate.
**Exercise 10**  
Cordblood DAT  
Recording Results

<table>
<thead>
<tr>
<th>Mom's Name and Hospital Number</th>
<th>Mom's Type &amp; Rh</th>
<th>DAT</th>
<th>Cell Typing with Anti-</th>
<th>Rhlg Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

*only if infant appears to be AB positive  
**for D neg infants

To answer RhIg yes/no:

1. Mom D neg, baby D neg, answer NO  
2. Mom D pos, answer NO  
3. Mom D neg, baby D pos, answer YES

Once you have determined which patient is an RhIg candidate, obtain maternal specimen from the instructor.
Exercise 10  Rh Immune Globulin (RhIg) Work

Study Questions

1. Describe how the D negative mother is exposed to the D antigen during the birth process. (1 point)

2. Define “kernicterus”. (1 point)

3. Describe the product “Rh Immune Globulin” (RhIg). (1 point)

4. State how administration of RhIg prevents sensitization of the mother to the D antigen and within what time frame it must be administered. (2 points)

5. State the whole blood and packed cell volumes that 1 vial of RhIg will cover. (1 point)

6. State the reason for administration of ante-partum administration of RhIg and when during the pregnancy this is administered. (1 point)
7. List three (3) groups of women who are NOT candidates for RhIg? (1.5 points)

8. List seven (7) circumstances when an Rh negative woman should receive RhIg? (3.5 points)
9. What group of tests constitute an RhIg workup and briefly state the purpose of each test performed. (3 points)

10. What is the term used for anti-D present in the maternal serum due to administration of RhIg? What is the term used for an anti-D present in a D negative person who has been sensitized to the D antigen? (1 point)

11. Briefly describe the principle of the Rosette test? (2 pts)

12. Briefly describe the principle of the Kleihauer-Betke acid elution test? (2 pts)

13. Why is the rosette test more sensitive and specific than the micro D? (1 point)
14. A Kleihauer-Betke acid elution test was done. The results are 50 fetal cells in 2000 adult cells seen. Calculate the dose of RhIg needed. Show your work. (1 point)

15. How is it determined whether the anti-D detected in the maternal sample is immune or passively acquired? (1 point)

16. For each of the following determine whether or not the Mother is an RhIg candidate. (3 Points)

<table>
<thead>
<tr>
<th>Maternal ABO/D</th>
<th>Maternal Antibody Screen</th>
<th>Infant ABO/D</th>
<th>DAT</th>
<th>RhIg Yes or No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 0 Neg</td>
<td>Neg</td>
<td>0 Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>b. A Pos</td>
<td>Neg</td>
<td>0 Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>c. A Neg</td>
<td>anti-C + -K</td>
<td>A Pos</td>
<td>Pos</td>
<td></td>
</tr>
<tr>
<td>d. 0 Neg</td>
<td>Neg</td>
<td>B Pos</td>
<td>Pos</td>
<td></td>
</tr>
<tr>
<td>e. A Neg</td>
<td>passively acquired anti-D</td>
<td>0 Pos</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>f. A B Neg</td>
<td>anti-D due to sensitization</td>
<td>A Pos</td>
<td>Pos</td>
<td></td>
</tr>
</tbody>
</table>