Name  Rh Immune Globulin Work Up  
(RhIgW)

Principle
After a D negative woman has given birth to a D positive child, a sample of her blood must be tested to determine if she is a candidate for Rh Immune Globulin (RhIg) prophylaxis. An ABO and D type (including weak D), antibody screen and rosette procedure are performed and she is considered a candidate if: 1) she is D negative and weak D negative, and 2) her serum does not contain immune anti-D. The weak D and rosette are performed to determine if an excessive amount of fetal cells have entered her circulation which would not be covered by one vial of RhIg. If the weak D and rosette tests are positive, a Kleihauer Betke acid elution stain must be performed so that the amount of bleed can be calculated and the appropriate number of vials given to protect her.

Clinical Significance
Rh Immune Globulin (RhIg) is human anti-D in a bottle which, after injection, will coat the D positive fetal cells in the maternal circulation. This causes them to be removed from her circulation. This causes them to be removed from her circulation before her immune system has a chance to recognize them as foreign and produce anti-D. One vial of RhIg contains 300 ug of anti-D which is sufficient to prevent sensitization by 30 mLs of fetal whole blood or 15 mLs of packed red blood cells.

In addition to D negative women who give birth to a D positive child, the following women who are D negative should also be evaluated for RhIg prophylaxis:

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
8. blunt force trauma to the abdomen
An antepartum dose of RhIg is routinely administered to all D negative women at the beginning of the third trimester (28 weeks) of pregnancy since it has been proven that RhIg failures (the women received RhIg, but at the next pregnancy had developed anti-D anyway) are due to small fetal-maternal hemorrhages occurring during the last trimester of pregnancy. This has caused a significant decrease of RhIg failures. The only problem is that the mother may have weakly positive screens at delivery. After it has been ascertained that she did receive antenatal RhIg another dose must be given post-partum.
Acceptable Student Result Range
Accurately perform and interpret all tests to determine RhIg candidacy.

Time Frame
45 minutes.

Specimen
Post-partum maternal clot

Reagents
Blood bank reagent rack
0.85% saline Rosette kit

Procedure
1. Prepare serum and cell suspension tubes as usual.
2. Label tubes for ABO/D type, D ctrl, antibody screen and three additional tubes labelled ROS POS, ROS NEG, and ROS patient initials for the rosette test.
3. Add reagent antiserums and cells, patient serum and cells to all tubes as described in previous type and screen procedure.
4. To the “ROS POS” tube add one drop of rosette positive control cells, to “ROS NEG” add one drop of rosette negative control cells and to the “ROS Patient” tube add one drop of patient cells. Add one drop of rosette antibody reagent to each of the three tubes, mix well and place in 37°C incubator.
5. Centrifuge type and screen tubes for 15 seconds. Read antibody screen tubes first, record reactions. Add two drops of albumin to the antibody screen and incubate at 37°C for 20 minutes.
6. Immediately read and record D and DC tubes. If negative, immediately plate in 37°C water bath.
   (NOTE: If patient is D positive, stop here and investigate. Patient should be redrawn. If new specimen still indicates she is D positive, it indicates she was mistyped at doctor's office and she is not an RhIg candidate.)
7. After incubation, centrifuge screens for 20 seconds, read and record reactions.

8. Wash antibody screen and D, D control and three rosette tubes three times with saline.

9. a) Add two drops of AHG reagent to the screens, D and D control tubes.
    b) Add one drop of well-mixed rosette indicator cells to each of the three rosette tubes. Then, add one drop of rosette enhancement media to the three rosette tubes.

10. Mix all tubes well and centrifuge for 15 seconds.

11. Read macroscopically and microscopically.

12. To all negative AHG tubes add one drop of check cells. Centrifuge 15 seconds. Agglutination must be obtained or repeat test.

Interpretation of Results
1. If antibody screen is positive, perform a panel study and identify antibody specificity. If anti-D, determine if it is immune or due to antenatal RhIg. If it is due to antenatal RhIg, an additional dose must be given. If the anti-D is immune to nature, she is not an RhIg candidate.

2. If the D and DC tubes remain negative throughout the procedure, she is an RhIg candidate. If she turns out to be D positive or weak D positive, she is not an RhIg candidate. If the D is weakly positive mixed-field and the DC is negative, this indicates a large feto-maternal hemorrhage not covered by a single vial of RhIg. A Kleihauer-Betke acid elution stain must be performed to quantitate the bleed and determine the number of vials of RhIg which must be given.

3. If the rosette on the patient is negative and the controls give appropriate results, this is indicative that one vial of RhIg will cover the bleed. If a positive reaction is obtained on the patient, this is indicative of an excessive fetal-maternal hemorrhage and a Kleihauer-Betke acid elution stain must be performed. If the controls do not give appropriate reactions, the entire test must be repeated.
### Rh and Rh Control/Microscopic D⁺

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### Fetal Screen/Rosette Test

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