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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- Explain the importance of detecting Rh-positive fetal cells in an Rh-negative mother.
- List the tests used to detect and quantitate fetal cells.
- Explain the basic principles of each test.

When an Rh-negative (D-) woman is pregnant with an Rh-positive (D+) fetus, there is a possibility that a small amount of D+ fetal cells will enter the maternal circulation during the normal course of the pregnancy and delivery. Some other instances that may cause this to occur are trauma to the mother’s abdomen, ectopic pregnancy, special medical procedures (amniocentesis), abortion, antepartum hemorrhage, or fetal death. The foreign D+ cells of the baby may cause the mother’s immune system to produce anti-D. Once the maternal anti-D is formed it can cross the placenta, which may cause problems to the fetus ranging from anemia to fetal death. The first D+ pregnancy is not usually affected, since it is during this pregnancy that the mother makes the anti-D; however, subsequent pregnancies are often affected. In order to prevent the formation of maternal anti-D, Rh Immune Globulin (RhIg) is administered to all D- women at 26–28 weeks of pregnancy and within 72 hours of trauma, special medical procedures, or abortion, since the Rh type of the fetus is unknown, as well as after the delivery of a D+ baby.

RhIg contains anti-D that attaches to D+ fetal cells in the maternal blood causing them to be removed from the maternal circulation. The mother’s immune system no longer has to produce anti-D to protect her from these foreign antigens and the RhIg ultimately protects the fetus and future pregnancies. The dosage of RhIg to be administered is determined by the amount of D+ fetal red cells in the maternal circulation. Normally, this is <15 ml (30 ml of whole blood), and one dose is sufficient. However, it is very important to screen the post-partum D- mother who delivers a D+ baby to determine if a large fetal maternal hemorrhage (FMH) has occurred.

The rosette test is a screening procedure, which easily detects the presence of D+ fetal red cells in the maternal circulation. To perform this test, a sample of the mother’s D- blood is mixed with commercial human source anti-D. The anti-D antibodies attach to any D+ fetal cells present. D+ indicator cells are then added to the mixture. If D+ fetal cells are present, the indicator cells will attach to the anti-D on the fetal cells and form “rosettes” around them. If rosettes are detected, a quantitative test should be performed to calculate the amount of FMH so that the proper dose of RhIg can be administered.
The Kleihauer-Betke (K-B) test is one method used to quantitate FMH. In the K-B test, a slide is made of the mother’s blood. The slide is then exposed to a citric acid buffer. The acid causes the adult hemoglobin to leach from the adult cells into the buffer while the fetal hemoglobin remains in the fetal cells. The slide is then stained with eosin. The fetal cells, regardless of Rh type, can be differentiated from the adult cells because the fetal cells easily adsorb the stain. The fetal cells are counted as a percentage of the total cells to determine the amount of FMH. From this amount the appropriate dosage of RhIg to be administered can be calculated. One dose of RhIg protects against 15 ml of fetal cells (30 ml of whole blood). The K-B test, though widely used, is very imprecise. Therefore, an additional dose of RhIg over the calculated dose is usually administered.

A more accurate method for calculating the amount of fetal cells in the maternal circulation is flow cytometry. This is a process where cells pass single file through a sensor that can measure individual characteristics to identify different kinds of cells. In this test, a fluorescent indicator is added to the maternal sample. The indicator, which may be directed against the D antigen or to the fetal hemoglobin, attaches to the fetal cells. The sample is then passed through a flow cytometer. The fluorescent fetal cells, which will form a population distinct from the maternal cells, can be counted (F cell count). This test is able to analyze thousands of cells in a very short time span, which will allow an accurate assessment of the FMH. The amount of RhIg to be administered can then accurately be determined. Although this test is very precise, it requires specialized equipment, which may not be available at all institutions or at all hours.
EDUCATIONAL COMMENTARY – FETAL CELL DETECTION AND QUANTITATION (cont.)

In summary, the administration of RhIg is important in D- pregnant women to protect fetuses of the current and future pregnancies. The detection of D+ fetal cells in a D- mother is very important in determining the amount of RhIg to be administered. The rosette test is a very simple screening test that, if positive, must be followed-up with a quantitative test. The flow cytometry test, while very accurate, is not widely available. The K-B test, while imprecise, provides a guideline for determining the amount of RhIg to administer. Usually, an additional dose of RhIg, which acts as a safety margin, is added to the K-B calculated dose to prevent undertreatment.

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