1. **What is the principle of the KB test?**
   Adult hemoglobin, but not fetal hemoglobin, is soluble in a citrate buffer with pH 3.2 and will elute out of the red cells.

2. **What is being detected?**
   The KB test detects red cells containing Hgb F that are resistant to acid elution (a.k.a. acid elution test for Hgb F).

3. **When is this test done?**
   Commonly done to detect the amount of transplacental hemorrhage at delivery to determine the prophylactic dose of Rh immune globulin to give an Rh (-) mother with an Rh (+) baby and positive FMH screen. Also done on Rh (+) or (-) mothers to detect fetal leakage into amniotic fluid or maternal circulation following a traumatic amniocentesis or other trauma.
   
   **Note:** The FMH screen detects Rh (+) red cells, not Hgb F.

4. **What is the usual patient specimen?**
   EDTA whole blood (maternal) or amniotic fluid; thin smears are made and allowed to air dry.

5. **Where do we obtain fetal cells for the positive control?**
   Positive control cells come from fresh cord blood or blood drawn from a normal newborn.

6. **What is used for the negative control?**
   The negative control is normal adult blood, usually from a male.

7. **Are controls always run?**
   Controls are always run to assure proper elution by the acid buffer (must be evenly distributed on slides). The positive control should have 10-20% fetal cells and the negative control should have no positive cells.

8. **List in correct order the following KB procedure steps:**
   1. Prepare patient & control smears
   2. Fix smears in 80% ethanol
   3. Incubate slides in citrate buffer
   4. Stain smears with erythrosine
   5. Validate control slides
   6. Count fetal cells on patient slide
5. Describe the appearance of positive staining cells. 
Red cells containing Hgb F stain bright pink due to erythrosin stain

5. What is the appearance of red cells containing Hgb A? 
Negative staining, adult red cells that contained Hgb A, appear as pale, ghost cells

5. How do white cells stain? 
White cells, such as lymphocytes, stain pink and must not be confused with fetal cells

How many fetal cells do you count in KB fields A, B, & C?

A: 7 (+) cells
B: 4 (+) cells
C: 5 (+) cells

6. If a total of 2050 RBCs are counted and 20 fetal cells are seen, what is the percent of fetal cells in maternal circulation?
Percent fetal cells = 20/2050 x 100 = 0.98%
One way to calculate the volume of fetomaternal hemorrhage is taking the percent fetal cells times 50 to obtain mL of whole blood. The ‘50’ represents the mother’s blood volume (5000mL). RhiG covers a 30 mL leak of whole blood and must be given within 72 hours of the bleed.

7. A Kleihauer, performed on an Rh negative mother with a positive FMH screen, shows zero fetal cells. Both controls ‘worked’. Could there still have been a fetal leak into maternal circulation?
A fetal leak could have occurred even though fetal cells were not seen on the Kleihauer (0.00%)

7. Would Rh immune globulin be given?
A standard dose of 1 vial of RhiG would still be given

8. Name several conditions that may show positive cells on the Kleihauer-Betke stain.
- Post-partum mothers following a transplacental hemorrhage
- Newborns/infants less than 6 months old
- Hereditary persistence of hemoglobin F (HPHF)...see SL page 129
- Disorders that compensate with hemoglobin F such as beta thal major or sickle cell disease

Note: Hgb F can be quantitated using flow cytometry

You are now ready to perform a Kleihauer-Betke count/interpretation on the case slides provided and report your results on the worksheet found in the Special Tests Unit of the Hemo II manual.