Kleihauer-Betke Acid Elution Stain

1. Prepare a control slide by mixing 1 part cord blood (Group O) with 9 parts normal adult blood (Group O). The cord blood confirms a positive result and the adult blood, a negative one. Thus, the control slide is both a positive and negative control. Do not select a specimen from a woman who is or was recently pregnant.

2. Place 3 drops of saline and 2 drops of maternal blood in an appropriately labeled tube. Place 3 drops of saline with 2 drops of control mixture in an appropriately labeled tube.

3. Label slides in pencil. Place one drop of diluted maternal blood or control near one end of labeled slide. Prepare thin film by drawing the edge of another slide through the drop of blood and across slide. Make at least 2 maternal and 1 control slide.

4. Air-dry the slides at room temperature. The slides should now be processed immediately throughout the entire procedure. It is recommended that the slides be viewed by 10X magnification to determine if a monolayer of cells has been obtained. If this has not been achieved, a new slide should be prepared.

5. Place the slides in a clean vessel containing sufficient fixing solution to cover them. Allow the slides to remain in the solution at room temperature for 5 minutes.

6. Remove the slides and rinse thoroughly in distilled H₂O. Allow the slides to drain dry.

7. Place the slides in a clean vessel containing sufficient buffer solution to cover them. Allow the slides to remain in the solution at room temperature for 8 - 10 minutes.

8. Remove the slides from the solution and immediately place in a clean vessel containing stain. Stain for 3 minutes.

9. Remove the slides from the stain and rinse thoroughly in distilled H₂O. Dry the slides at room temperature.

10. The slides may be examined using high/dry (20X or 40X) power. The slides should not be examined with oil. Fetal cells will stain dark reddish-pink while adult cells will appear like “ghost cells.” The control slide must contain representative fetal and adult cells to confirm the staining process.

11. Count at least 2000 cells and record both the number of fetal and adult cells observed. Compare both maternal slides to be sure there is agreement between the two slides. If there is not agreement, prepare new maternal slides being sure to mix specimen well.

12. If the slide is positive for fetal cells have a second technologist confirm the results. There should be agreement between both techs for the percentage of adult and fetal cells.

13. Calculate percentage of fetal cells.

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\text{\%Fetal Cells} = \left( \frac{\text{# of fetal cells counted}}{\text{total # of cells counted}} \right) \times 100
\]

Example:

Kleihauer-Betke reported 1.3% fetal cells

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1.3 \times 50 = 65 \text{ mL of fetal whole blood}
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(The value of 50 is obtained by using 5000 mL—arbitrarily assigned mother’s blood volume divided by 100, from percentage)
14. Calculate dose of RhIG required.

**Dose computation:**
Fetal whole blood volume in mL/30 mL (amount of Rh-positive fetal blood that one 300 µg of RhIG will protect against)

Example:
65/30 = 2.2 doses of RhIG required

Because quantitation by this procedure is inherently imprecise and because the consequences of under treatment can be serious, it is desirable to provide a safety margin. One approach is as follows:

- When the number to the right of the decimal point is less than 5, round down and add one dose of RhIG (example: calculation is 2.2 doses, give 3 doses).
- When the number to the right of the decimal point is 5 or greater, round up to the next number and add one dose of RhIG (example: calculation is 2.8 doses, give 4 doses). Not more than 5 doses of RhIG should be given at one time. For large quantities, injections may be spaced over a 72-hour period for patient comfort.
- **Adding one vial to the calculated total is the transfusion service practice of Metropolitan City Hospital.**

**Notes**

1. Fetal hemoglobin resists elution from red cells under acid conditions, whereas adult hemoglobin is eluted. When a thin blood smear is exposed to an acid buffer, hemoglobin from adult red cells is leached into the buffer so that only the stroma (ghost cells) remains; fetal cells retain their hemoglobin and can be identified by a positive staining pattern (dark reddish-pink).

2. When a Kleihauer-Betke (K-B) test order is received, call the nursing unit to determine the reason for the test and the gestational age.

3. A Kleihauer-Betke test should be performed only for trauma or if the mother is D positive or D negative with a suspected fetal-maternal bleed.

4. White blood cells may take up the stain in varying degrees, but less than the fetal cells.

5. Hematological disorders in adults may produce increased levels of fetal-type cells.

6. The degree of elution of the adult hemoglobin may vary from patient to patient.

7. Normal adult blood contains less than 1.0% of fetal-type hemoglobin.

8. If no fetal cells are seen, report <0.1% fetal cells. If 1 or more fetal cells are seen, count cells and calculate % fetal cells. If the count is <0.1% fetal cells then report as <0.1% fetal cells. If the count is 0.1% or greater, report the actual percentage counted.

9. A K-B test may be performed to determine whether the neonatal specimen contains fetal or maternal blood or a combination of the two. The results will aid in determining the neonate’s blood type.