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Area	Laboratory-Blood Bank
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Acid Elution by Kleihaeur Betke Method - Dearborn Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide policies and instructions that will enable the Blood Bank staff to perform the Acid Elution/ Kleihauer Betke (KBT) quantitative fetal stain test using the Fetal Stain Kit provided by Simmler Inc. ®.

II. CLINICAL SIGNIFICANCE:

A. A quantitative fetal assay is required in cases where a significant volume of fetal blood has been suspected of entering the maternal circulation as indicated by a positive fetal bleed screening test (rosette test).

B. If either the maternal or neonatal RBCs are known to be weak D positive or partial D positive.

C. If the Rh type of either the fetal or maternal RBCs is undetermined for any reason.

D. If there is evidence of trauma in pregnancy and delivery has not occurred or if the pregnancy is greater than 18 weeks gestation.

III. PRINCIPLE

The test is based upon the resistance of fetal hemoglobin to elution by the eluting fluid. This resistance is contrasted to adult hemoglobin, which is easily eluted from the erythrocyte. 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 remains. Fetal cells retain their hemoglobin and can be identified by a positive staining pattern. The approximate volume of fetomaternal hemorrhage can be calculated from the percentage of fetal red

cells in the maternal blood film.

IV. INDICATIONS FOR TESTING:

A. The Acid Elution (KBT) test is an acceptable alternative for the preferred flow cytometry Fetal RBC Assay . The acid elution (KBT) stain should be ordered whenever there is a possibility that flow cytometry results will not be available within 48 hours of order. i.e. weekends and holidays.

B. Medical Director approval must be obtained in situations where the acid elution (KBT) stain is specifically requested by ordering physician when the results for Fetal RBC Assay will not meet STAT time requirements.

C. Blood Bank staff will communicate with Flow Cytometry and Hematology if appropriate to determine which test will be performed based on staffing, weekends, holidays, etc.

V. SPECIMEN COLLECTION AND HANDLING:

A. The test procedure requires a blood specimen collected from the mother after delivery of all products of conception. It is best to wait about an hour after delivery to allow any fetal RBCs to mix thoroughly in the maternal circulation, but the sample should be collected as soon as possible thereafter.

B. The preferred sample is a 6ml EDTA sample with affixed identifying label. Refer to Transfusion Medicine policy, Triaging And Identifying Acceptable Samples For Testing.

C. Do not use grossly hemolyzed specimens for testing.

D. If a delay in testing occurs, the sample may be stored at 1 – 10 ° C.

VI. REAGENTS:

A. Simmler Inc. Fetal Cell Stain Kit:

1. Fetal Cell Fixing Solution (80% Reagent Alcohol)
2. Fetal Cell Buffer Solution (Citrate Buffer 0.081M)
3. Fetal Cells Stain (Erythrosin-B, Fast Green)

B. Blood Bank Isotonic Saline

VII. SUPPLIES:

A. Test tubes, 12 x 75 mm are preferred

B. Disposable pipettes

C. Microscope

D. Microscope slides

E. Centrifuge

F. Staining Rack

VIII. QUALITY CONTROL (QC):

A. The positive and negative controls should be tested in parallel with each batch of patient samples as a qualitative confirmation of the stain performance.

B. A positive control may be prepared from a mixture of 1-drop cord blood and 9-drops adult male blood. Fetal cells will stain a dark reddish-pink while adult cells will appear white to light pink with a darker center.

C. A negative control may be prepared from adult male blood. It will show unstained (ghost cells) red blood cells.

D. The results are documented on the *Acid Elution/Kleihauer Betke Stain Quality Control Log.*

IX. PROCEDURE:

A. Stain Procedure

1. Prepare control samples as directed in Quality Control section above.
2. Mix the samples well by gentle inversion.
3. Dilute each control, place 3 drops of 0.85% saline and 2 drops of control sample into a properly labeled glass test tube. Mix by gentle agitation.
4. Place 1 drop of appropriate diluted control on a labeled glass slide near one end. Prepare thin film by drawing the edge of another slide through the drop of blood, and across the slide.
5. Mix the patient sample well by gentle inversion.
6. Place 3 drops of 0.85% saline and 2 drops of maternal blood into a glass test tube. Mix by gentle agitation.
7. Place 1 drop of diluted blood on a glass slide near one end. Prepare thin film by drawing the edge of another slide through the drop of blood, and across the slide.
8. Air-dry the slides at room temperature.
   NOTE: The slide should now be processed immediately throughout the entire procedure.
9. Squirt sufficient fetal cell fixing solution from the bottle to cover the blood smears. Allow the solution to remain on the smears at room temperature for 5 minutes.
10. Rinse thoroughly with tap water. Allow the slides to drain dry.
11. Squirt sufficient Fetal Cell Buffer Solution from the bottle to cover the smears. Allow the solution to remain on the smears at room temperature for 8-10 minutes.
12. Drain the buffer solution from the slides. Do not rinse. Immediately squirt sufficient Fetal Cell Stain from the bottle to cover the smears. Stain for 3 minutes.
13. Rinse thoroughly with tap water. Dry the slides at room temperature.
14. Slides may be examined by using either a dry or oil-immersion lens.
15. Slides should be read within 24 hours.

B. Counting with Miller Disk

1. Each slide is observed microscopically using 40x magnification and the Miller Disc eyepiece.
2. Count all adult cells in square B in successive, evenly distributed fields until a total of 222 red cells have been counted. At the same time, count the number of fetal cells in square A (includes square B).

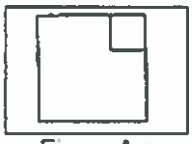

Figure A.

3. Calculate the % fetal cells as follows:
   % of fetal cells = $\frac{\text{\# fetal cells counted}}{20}$
   This calculation is based on the fact that square B in the Miller Disc is 1/9 the area of square A. In effect, counting 222 cells in square B will give a proportional count of 1998 (~2000) cells for square A.
4. Determine the Rhogam Dosage by accessing the College of American Pathologist) RhIg Calculator. This calculator may be applied for postpartum or antepartum RhIG assessment.
   a. Open the RhIG Calculator on the Sharepoint drive.
   b. Do not enter the mother's height or weight; the mother's blood volume is assumed to be 5000 mLs.
   c. Enter the percent fetal blood and press <Enter>.
   d. The recommended number of RhIG vials is calculated.
5. If the CAP calculator is not available the blood bank will supply the number of RhIG vials as calculated by the following:
   a. Calculate the ml. in whole blood of fetal-maternal bleed, multiply % fetal cells by 50.
      Ex. ml bleed = % fetal cells x 50
   b. Divide the fetal-maternal bleed in ml. by 30 to calculate the dose of Rhogam to be given.
      Ex. 45 ml. bleed/30 = 1.5
   c. Quantification by this procedure is inherently inaccurate and because the consequences of undertreatment can be serious, it is desirable to provide a safety margin in calculating RhIG dosage. Please use these additional steps to ensure proper dosage:
      i. When the number to the right of the decimal point is less than 5, round down and add one dosage of RhIG (ex. 2.2 doses, give 3 doses).
      ii. 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 (ex. 2.8 doses, give 4 doses).
6. No more than 5 doses of RhIG should be injected intramuscularly at one time. If more than 5

doses are required, please confirm with the Medical Director before proceeding.

X. INTERPRETATION:

A. Fetal cells will stain a dark reddish-pink while adult cells will appear white to light pink with a darker center. Staining intensity of adult cells may vary slightly within lots of reagents; however, fetal and adult cells will be easily differentiated.

B. Both controls must perform as expected before an interpretation can be made.

C. Acceptable results for Obstetric patients is less than 0.3%.

D. If fetal cell % is 0.3% or greater, confirm with a second technologist's count. Notify Blood Bank supervisor of any patient with 0.3% or greater result.

E. If the bleed is greater than 2.25% the Medical Director must be notified for further instructions.

XI. RESULT REPORTING:

A. The result is reported as a % in the comment field in Beaker. i.e. 0.5%

B. To report a negative result, enter 0.0%

XII. SPECIAL NOTES:

A. If there is a question regarding the need for additional Rhogam, it is preferable to administer another dose to prevent the risk of undertreatment.

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

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

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

E. Lymphocytes may take up stain in varying degrees, but less than fetal cells.

XIII. REFERENCES:

1. AABB Technical Manual, current edition.
2. Package Insert, Simmler Fetal Stain Kit rev 03/2017.

Attachments

Acid Elution by Kleihauer Betke Method Quality Control Log

Approval Signatures

Step Description	Approver	Date

	Jeremy Powers: Chief, Pathology	5/27/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	5/25/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	5/25/2022
	Kimberly Geck: Dir, Lab Operations B	5/25/2022
	Kelly Sartor: Supv, Laboratory	5/25/2022
	Kelly Sartor: Supv, Laboratory	5/25/2022

Beaumont HEALTH

Beaumont Laboratory
Dearborn, MI

Acid Elution by Kleihauer Betke Method
Quality Control Log

Fetal Cell Fixing Solution Lot # / Expiration Date _____

Fetal Cell Buffer Lot # / Expiration Date _____

Fetal Cell Stain Lot # / Expiration Date _____

Date	*Positive Control Acceptable ?* *Circle One*	*Negative Control Acceptable ?* *Circle One*	*Tech ID/ Initials*	*Review Date /By*
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
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	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		
	Yes / No	*Yes / No*		

Positive and Negative controls must be tested in parallel with each batch of patient testing.