Sutter Health
Sutter Roseville Medical Center

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Owner:	*Alex Alba: Spvr, Laboratory*
Policy Area:	*Lab - Hematology*
References:	
Applicability:	*Sutter Roseville Medical Center*

Performing the Kleihauer-Betke Fetal Hemoglobin Assay

PURPOSE

This procedure describes how to identify fetal red cells in the presence of adult red cells in maternal blood to estimate fetal-maternal hemorrhage and determine dosage of RhIG for post partum immunization.

POLICY

- The KB Stain should be ordered in the following instances:
  - Rh negative women following delivery of Rh positive baby
  - After potentially sensitizing events in Rh negative women after 20 weeks gestation such as abdominal trauma, intrauterine death, miscarriage, etc
- The test is performed by a CLS
- Expected TAT is 60 minutes from receipt of sample
- Order code is KBST

EQUIPMENT, REAGENTS AND SUPPLIES

NOTE: Do not mix kit components from different lots

- Sure Tech Citrate Buffer (0.081 M, pH 3.0-3.5)
- Fixing Solution (80% Ethyl alcohol v/v)
- Sure Tech Hemoglobin (HGB) Staining Solution
- Microscope
- Microscope slides
- 12x75mm plastic test tubes
- Staining and drying racks

SPECIMEN REQUIREMENTS

- 1mL maternal whole blood anticoagulated with EDTA
- Specimen should be collected as soon after delivery as possible
- Specimen is stable for 6 hours post-collection

QUALITY CONTROL PREPARATION

Quality control slides are to be made with each patient test as described below:

- **Negative** Control
  - 6-8 drops of 0.85% saline
  - 2 drops of normal adult male EDTA blood
- **Positive** Control
  - 3 drops of 0.85% normal saline
  - 1 drop of normal male whole blood in EDTA
  - A small amount of untransfused newborn blood from an EDTA microtainer
    - Place 2 wooden applicator sticks into microtainer then transfer the blood adhering to the sticks to the saline/adult blood mixture

QUALITY CONTROL INTERPRETATION

The control slides must show the following expected reactions or the procedure is invalid and must be repeated:

- **Negative Control**
  - adult red blood cells will appear as ghost cells due to no reaction with the stain
- **Positive Control**
  - fetal HGB in red blood cells will react with the stain and stain bright red
- **WBC and Platelets**
  - WBC's will stain pink but can distinguished from fetal RBC's by their nucleus and granular appearance and must not be counted as fetal RBC's
  - Platelets will stain pink but are smaller with spike like projections and must not be counted as fetal RBC's

PROCEDURE

Follow the instructions below to perform the Kleihauer-Betke stain:

Step	Action
1	Label (3) 10 X 75 plastic tubes as patient, positive control, and negative control.
2	Add 3 drops of 0.85% saline to the patient and positive control tubes. Add 6-8 drops of 0.85% saline to the negative control tube.
3	Add 2 drops of well mixed patient's blood to the appropriate tube and prepare the control dilutions as described then mix well.
4	Make 2 – 3 thin smears of each dilution and label appropriately. Air dry at room temperature
5	Place the slides face up on the staining rack in the Hematology sink and cover each slide with the fixing solution. Allow the slides to fix for 5 minutes.
6	Tilt each slide to allow the fixing solution to drain off and rinse thoroughly with DI water. Transfer to drying rack and allow to air dry.
7	Replace the slides on the staining rack and cover with the citrate buffer solution. Allow to remain for 10 minutes.
8	Tilt each slide to allow the citrate buffer solution to drain off. Blot excess buffer from each slide

	and cover immediately with the HGB staining solution. Allow the slides to stain for 3 minutes.
9	Tilt each slide to allow the HGB staining solution to drain off and rinse thoroughly with DI water. Transfer to drying rack and allow the slides to dry at room temperature. **Read within 24 hrs.**
10	Using the 100X oil lens at the feathered edge of the slide, count all fetal cells contained in the large square area of the Miller Ocular. • This includes fetal cells in the smaller square. • Be sure to count all fetal cells touching the left and upper boundary of the square but do not include the fetal cells touching the right and lower boundary.
11	Keep track of the total number of RBC's in the small square as well. • Be sure to include all RBC's touching the left and upper boundary of the square but do not include the RBC's touching the right and lower boundary. • Continue examining fields at random until 150 RBC's have been counted in the small square. NOTE: The appearance of the negative and positive QC will be documented but the number of fetal and adult red blood cells does not have to be counted
12	Document the QC and patient results on Form A: KB Stain Result Log.
13	Enter the QC and patient results in the LIS • Follow the Detecting and Correcting Data Entry Errors procedure when reviewing manually entered tests

% FETAL CELLS CALCULATION

$$\%\ Fetal\ Cells = \frac{Total\ Fetal\ Cells\ in\ large\ square}{Total\ RBCs\ in\ small\ square\ x\ 9} x\ 100$$

INTERPRETATION

- Fetal cells will stain bright red while adult red blood cells will appear as ghost cells with white to light pink with a dark center.
- WBC's and PLT's will stain pink and must be distinguished from fetal cells and not be identified as fetal cells.

REPORTING RESULTS

- Function: **MEM**
- Worksheet: **RVHM2**
- QC Codes:
  - C-KBSTN (Negative QC) - enter 0 for negative
  - C-KBSTP (Positive QC)- enter 1 for positive
- Patient Result Fields:
  - FETLCT- Fetal cells counted: Enter the number of fetal cells counted
  - ADULTC – Adult Cells Counted: Enter the number of adult cells counted
  - FETPCT- % Fetal Cells: the LIS will perform a calculation for the %Fetal Cells
  - VOLFB- Volume of Fetal Bleed: Do not report this result. It is set to auto HIDE

REFERENCE RANGE

≤ 0.3 %

CRITICAL VALUE

None

TECHNICAL LIMIT

None

PROCEDURAL NOTES

- Blood smears from adults typically have only ghost cells but occasionally <1% of cells containing Hb F may be found in persons who are presumably normal.
- Other conditions which have been associated with increased fetal hemoglobin containing cells include hereditary persistence of fetal hemoglobin, thalassemia minor, leukemia, sickle cell anemia, pernicious anemia, aplastic anemia and other conditions involving hemopoietic stress as well as several chromosomal abnormalities.

RELATED DOCUMENTS

Valley Laboratories procedure, *Detecting and Correcting Data Entry Errors*

REFERENCES

- Package Insert: Fetal Cell Stain kit, Screening test for the detection of Erythrocytes containing Fetal Hemoglobin in Maternal Blood

All revision dates: 11/10/2021

Attachments

KB Stain patient result log.doc

Approval Signatures

Step Description	Approver	Date
Medical Director	Lindsey Westerbeck: Dir, Lab	11/10/2021
Laboratory Director	Lindsey Westerbeck: Dir, Lab	11/5/2021