Special Hematology/ 111 FETAL CELL STAIN (KLEIHAUER-BETKE)

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Initial version

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Periodic review	Lab Director	4/2/2024	1.0	Ali Mousa Ramadan MD	
Periodic review	Medical Pathologist	3/22/2024	1.0	Lekidule Taddesse-Heath	
Approval	Lab Director	8/15/2021	1.0	Ali Mousa Ramadan	
Approval	Laboratory Operations Manager	8/13/2021	1.0	Wendell McMillan	
Approval	Quality Coordinator	8/1/2021	1.0	Lorraine Foster	Initial electronic version

Version History

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1.0	Approved and Current	Initial version	7/22/2021	8/15/2021	Indefinite

HOWARD UNIVERSITY HOSPITAL
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

STANDARD OPERATING POLICY AND PROCEDURE MANUAL

Special Hematology/111 **FETAL CELL STAIN (KLEIHAUER-BETKE)**

PURPOSE:

Adult hemoglobin (HBA) is rapidly dissolved from erythrocytes at 20-25C in air dried and alcohol fixed smears with alcoholic ferric chloride/hematoxylin solution at a pH of 1.3-3.6. Fetal hemoglobin (HBF), however, is only slowly eluted. Treating eluted slides results in the selective staining of fetal hemoglobin-containing erythrocytes. The demonstration of fetomaternal microtransfusion is important in the prevention of Rh sensitization. In hemorrhage due to placenta previa, the method provides a rapid indication as to whether, and to what extent, the fetus is losing blood. The Kleihauer-Betke is recommended, to be done on maternal blood after delivery, to estimate the volume of fetal-maternal hemorrhage.

REAGENTS:

- Fetal Cell Solution (805 Reagent Alcohol)
- Fetal Cell Buffer (Citrate Buffer, 0.081M)
- Fetal Cell Stain (Erythrosin-B, Fast Green)
- 0.85% Saline Solution

EQUIPMENT AND SUPPLIES

- Timing Device
- Microscope Slides
- Microscope
- Pipette
- Glass Test Tube
- Coplar Jar or Containers for Fixing, Staining

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SPECIMEN:

Maternal blood collected with the aid of EDTA or oxalate should be used. Samples should be stored at 2-8° C until assayed. Freshly drawn blood should be used for this test. If the test cannot be performed immediately, the slides should be made, fixed and stored at room temperature.

AMNIOTIC FLUID:

The presence of fetal erythrocytes in amniotic fluid may be assessed by substituting the fluid for blood in the procedure.

CORD BLOOD:

Cord blood is fetal cells and is not suitable for assessing fetal/maternal hemorrhage. Cord blood should be used for the preparation of positive control.

QUALITY CONTROL:

- A positive and negative control should be prepared with each patient sample.
- Positive control slides should be made by mixing 1 part of cord blood to 9 parts of normal adult male blood.
- Negative control slides should be made from a male specimen with normal CBC results.
- All positive controls slides must contain fetal cells in order to validate the test results.
- If there are no fetal cells on positive slides and/or the negative slides contain fetal cells, all other results are invalidated. Repeat test

PROCEDURE:

1. Mix the blood sample well by gentle inversion.
2. Place 3 drops of 0.85% saline and two drops of maternal blood into a glass tube. Mix by gentle agitation.
3. Place one 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.
4. Air dry the slide at room temperature.

NOTE*:* The slide should be processed immediately throughout the entire procedure. It is recommended that the smear be viewed at 100X magnification to determine if a monolayer of cells has been obtained. If it is not achieved, a new smear should be prepared.

5. Place the smears in a clean vessel containing sufficient FETAL CELL FIXING SOLUTION to cover the smears. Allow the smears to remain in the solution at room temperature for 5 minutes.
6. Remove the smears and rinse thoroughly in distilled water. Allow the slides to drain dry.
7. Place the smears in a clean vessel containing sufficient FETAL CELL BUFFER SOLUTION to cover the smears. Allow to remain in the solution at room temperature for 8-10 minutes.
8. Remove the slides and immediately place in a clean vessel containing FETAL CELL STAIN. Stain for 3 minutes.
9. Remove the slides from FETAL CELL STAINING SOLUTION and rinse thoroughly in distilled water. Dry the slides at room temperature.
10. Slides are examined using 40X magnification. Fetal cells will stain 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. The slide should be read within 24 hours.

NOTE: Reticulocytes may stimulate fetal hemoglobin cells since they stain light red after elution. They can, however, be distinguished from fetal hemoglobin cells by their fine granular structure.

Save all slides including positive for the pathologist review.

INTERPRETATION/RESULTS:

The common means of reporting fetal cells is as a percentage of normal adult cells. This ratio is achieved by randomly observing 8-10 fields at 40X magnification.

1. Count 1000 red cells and record the number of fetal cells noted within the fields.
2. Determine the percentage of fetal cells by dividing the total number of fetal cells counted by the number of adult cells counted.

CALCULATION:

Results are reported as a percentage of fetal cells:

$$\% \text{ OF FETAL CELLS} = \frac{\text{\# OF FETAL CELLS X 100}}{\text{\# OF RED CELL (2000 adult cells)}}$$

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RhIG DOSAGE CALCULATION:

The number of doses of RhIG is determined by dividing the estimated volume of fetal blood present by 30

For example:

1. Kleihauer-Betke test result reported as 1.3%
2. (1.3/100) x 5000ml* = 65ml of fetal blood
3. 65/30ml per dose = 2.2 doses of RhIG

*= mother's arbitrary assigned volume

It is desirable to provide a safety margin in calculating RhIG dosage. One approach is as follows;

1. When the number of the right of decimal point is less than 5, round down and add one dose of RhIG (example: if the calculation comes to 2.2 doses , give 3 doses)
2. 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: if the calculation comes 2.8 doses, give 4 doses)

Not more than five doses of RhIG should be injected intramuscularly at one time. For larger quantities, injections can be spaced over 72-hours period for the patient's comport; an optimal time sequence has not been established. The intravenous preparation of RhIG can be used when higher doses are required. According to the package insert, a maximum dose of 300IU should be given each injection, every 8 hours, until the total calculation dose has been administered.

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Rho GAM DOSAGE:

% Fetal cells	Vials of RhIG to inject	Dose	
		In µg	In IU
0.3 - 0.8	2	600	3000
0.9 - 1.4	3	900	4500
1.5 - 2.0	4	1200	6000
2.1 - 2.5	5	1500	7500

ENTERING RESULTS IN THE NOVIUS COMPUTER

1. After signing on, activate TESTS by clicking once.
2. Enter the number of the sample to be entered in the upper left hand corner or be searching for the number in the listed order.
3. Activate the ENTER INDIVIDUAL RESULTS Button, and then enter the results.
4. Select COMP/SAVE box to file results.

REFERENCES:

1. Kleihauer, E., Brown, H. Bethe, K., Klin Wochenschrift 35:637 1968.

2. Clayton, E.M., Feldhaus, W.D., Clin. Path. 40:487 1963.

3. Oski, F.A. Naiman, J.L., Hematologic problems in the newborn. 2nd. Saunders, Philadelphia, PA, 1972, pg. 62-63.

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