KB STAIN FOR FETOMATERNAL HEMORRHAGE

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PURPOSE

To provide instructions for the performance of the acid stain for fetomaternal hemorrhage, also known as the Kleihauer Betke staining test.

BACKGROUND

The Kleihauer Betke (KB) stain distinguishes fetal hemoglobin from adult hemoglobin, and can therefore be used to assess the extent of fetal cell transfer into the maternal circulation. It is used for the purpose of:

1. Quantifying the amount of Rh Immune Globulin needed to deter maternal sensitization
2. Detect fetal bleeds after trauma.

The KB stain is to be performed whenever a positive result is obtained with the Fetal Screen for Fetomaternal Hemorrhage. It is also used when prenatal trauma has occurred to the mother – a fall or accident, for instance – where there is no infant sample available to determine the infant's Rh type.

RELATED DOCUMENTS

J-W-TS0251	Fetal Bleed Screening Test
J-F-TS1045	KB Stain QC Form

SPECIMEN COLLECTION AND STORAGE

- Maternal 7 ml EDTA sample drawn as soon as possible after delivery or potential sensitizing event. Samples should be stored at 2-8C until assayed and can be stored at this temperature for up to two weeks. Specimens should be assayed as promptly as possible. Hemolyzed specimens are not acceptable for use.
- Amniotic and other body fluids will need to have the red cells isolated by washing with saline and adding protein (albumin) to the final suspension.

REAGENTS AND EQUIPMENT

Sure-Tech Fetal Hemoglobin Kit
- Red Cell Fixing Solution (Ethanol 80%)
- Citrate/Phosphate Buffer (0.2 mol/L)
- Hemoglobin Staining Solution (Erythrosin 0.1%)

Thermometer – kept on bookcase that holds component modification labels
0.85% Saline
Coplin Jars
Slide Holder
Microscopic slides, frosted end
Microscope, 40x dry objective
12 x 75 mm glass test tubes

***Note*:** Reagents are stored between 20-25C and are stable for the period indicated on the label. Reagents may be reused until deterioration in the quality of the slides is noted. Do not pour used reagent back into the original containers. Pour them into "working" bottles.

QUALITY CONTROL

Record results on the KB Stain QC Form.

- High Positive Control: Add 0.1 ml cord blood to 0.9 ml normal adult male blood
- Low Positive Control: Add 0.05 ml cord blood to 0.95 ml normal adult male blood
- Negative control: Normal adult male blood

Notes:
1. Fixed slides are stable for up to 30 days stored in the freezer.
2. Control slides may be prepared ahead of time and stored in the freezer. They must be allowed to come to room temperature before starting the procedure. Begin with step 10. Do not re-fix!

TEMPERATURE

The reagents for this test must be at room temperature before using. Historically that temperature range for clinical labs has been approximately 25C ± 2C. With the increased use of automated instrumentation, the temperature in the lab may be significantly less than what was once considered room temperature. If the KB stain is performed at lower temperatures, it may adversely affect the test. It will be necessary to take the ambient air temperature in the location where the test will be performed prior to running the test. If the temperature is < 23C, then the following adjustments must be made:

Ambient 20 – 23C	Ambient <20C
Citrate Buffer must be adjusted to 23-27C	All three reagents adjusted to 23-27C

Temperature of the reagents may be adjusted by using a warm water bath or a light bulb until the desired temperature is reached.

Note: Actually, the test may be run at any temperature providing sufficient quantity of adult hemoglobin is eluted from the adult cells in the specified period of time, causing adult cells to stain a light pink in contrast to the fetal cells (dark reddish-pink).

INSTRUCTIONS

1. Mix patient sample and controls gently.

2. Place 3 drops of 0.85% saline and 2 drops of blood (patient and controls) in properly labeled test tubes and mix gently.

3. Make 1 smear of each positive control, 2 smears of the negative control, and 4 smears of the patient sample, by placing 1 small drop of diluted blood near the frosted end of the slide and making a thin smear, leaving a monolayer of cells.

4. Air dry the slides for 15 minutes at room temperature.

5. Record the ambient air temperature on the KB Stain QC Form

6. Adjust the temperature of the Sure-Tech reagents, if necessary, as per the above table.
7. Place the dried slides in a Coplin jar containing enough Red Cell Fixing solution to cover the smears. Raise and lower the slides 2-3 times to coat the slides in solution. Let remain in the fixative at the appropriate temperature for 5 minutes.

8. Pour fixative solution from the Coplin jar into the “in use” fixative plastic bottle, using a funnel.

9. Rinse slides thoroughly in deionized water, filling and emptying the Coplin jar at least 5 times, then let the slides air dry for 10-15 minutes at room temperature.

10. Place the dry slides in a Coplin jar containing sufficient Citrate/Phosphate Buffer to cover the smears. Raise and lower the slides 2-3 times to coat, and let remain in the buffer at the appropriate temperature for 10 minutes.

11. Pour buffer from the Coplin jar into the "in use" buffer plastic bottle, using a clean funnel.

12. Remove slides from jar and blot excess buffer from the slides.

13. Place the wet slides in a Coplin jar containing sufficient Hemoglobin Staining Solution to cover the smears. Raise and lower the slides 2-3 times to coat. Let slides remain in the stain for 3 minutes.

14. Pour stain solution from the Coplin jar into the "in use" stain bottle through a clean funnel.

15. Rinse the slides in deionized water, filling and emptying the Coplin jar at least 5 times.

16. Allow the slides to dry at room temperature.

17. Slides must be examined by using oil immersion.
    - Fetal cells will appear a dark reddish-pink while adult cells will appear light pink with a slightly darker center. Other cells may also stain to a varying degree and these cells must be identified so as not to be counted as fetal cells.
    - Adult cells will appear whit to light pink with a slightly darker center
    - Platelets will stain pink but are usually smaller with spike-like projections.
    - Lymphocytes will stain pink but can be distinguished from fetal cells by their granular appearance.

    Note: Refer to the Sure-Tech Diagnostics Kleihauer-Betke Fetal Hemoglobin Reference Manual for assistance in fetal cell identification.

18. Count the total number of adult and fetal erythrocytes in as many fields as required to give a total count of at least 2000 cells.

19. Reagents in use will be stored in marked "in use" bottles to prevent evaporation. Mark the lot number and expiration date on the bottle.

20. Keep slides for one week.

INTERPRETATION OF RESULTS

Results are expressed as % Fetal Cells. AABB recommends the following calculation be used.

1. Calculate the percent fetal cells in the total counted.

   Example: Total RBCs counted = 2160
   Total Fetal RBCs counted = 10
   % Fetal Cells = $\frac{10}{2160}$ x 100 = 0.46%

2. To determine the number of vials of Rh Immune Globulin necessary to protect against Rh sensitization, you may use the ***RhIG calculator*** or the chart below.

Percentage of fetal cells in circulation	Packed cell volume of FMH	Vials of RhIG indicated
0.0 – 0.29%	< 15 mL	1
0.3 – 0.89%	15 -- 44.9 mL	2
0.9 – 1.49%	45 -- 74.9 mL	3
1.5 – 2.09%	75 -- 104.9 mL	4
2.1 – 2.69%	105 --134.9mL	5

Should the percentage of fetal cells in the maternal circulation equal or exceed 2.7% (which can occur when a chronic fetal-maternal bleed has been present), additional RhIG beyond what is shown in the chart above will be needed to prevent Rh sensitization. Use the ***RhIG calculator*** to determine the amount of RhIG which must be administered

3. The ***RhIG calculator*** obtained from the CAP is located on the desktop of each computer in the transfusion service. It is to be used as follows:
   - Click on the icon to open the calculator
   - Do not enter information in the mother's height or mother's weight boxes as that information is not given to the transfusion service with the order.
   - Type in the percent fetal blood as calculated by the KB stain.
   - The volume of bleed will be automatically calculated
   - The recommended number of vials to give will appear in the last field.

CERNER ORDERING AND RESULTING

1. Cerner order code is KB STAIN.

2. Enter results under SRE by entering F11, See Com, then PF1, then G, and one of the following, filling in the blanks of the template as appropriate:
   - *Rh Negative Pregnant Women*
     - **KBRG** is used when:
       - After delivery of an Rh positive infant to an Rh negative mother. RhIG is always indicated, and dosage must be determined by the KB Stain results
       - Prenatal trauma has occurred to the mother. Infant Rh type must be assumed to be Rh Pos. RhIG is always indicated, and dosage must be determined
   - *Rh Positive Pregnant Women* – to determine if a fetal bleed due to prenatal trauma has occurred so that appropriate steps for patient care can be taken by the physician.
     - **KBNEG** to be used when there are no fetal cells seen.
     - **KBPOS** to be used when fetal cells are seen

INSTRUCTIONS FOR AMNIOTIC AND OTHER BODY FLUIDS

1. The presence of fetal erythrocytes in amniotic and other body fluids can be accessed by substituting the fluid for blood in the above listed procedure.

2. Isolate the cells by centrifugation.

3. Wash the cells 3 times with saline.

4. Resuspend the cells and add 1-2 drops of 22% albumin.

5. Follow the instructions listed for smear preparation steps 3-14.

6. Report results as fetal cells seen or not seen, See Cerner ORDERING AND RESULTING SECTION.

PROCEDURAL NOTES

When testing fluids other than EDTA sample, access the sample and determine number of saline washes to get clean cells. Saline washes remove extraneous protein from sample, the addition of 22% albumin to sample after washing helps the cells adhere to the slide for staining.

PROCEDURAL LIMITATIONS

Cord Blood is fetal blood and is not suitable for assessing fetal/maternal hemorrhage. Cord blood is used for the preparation of the positive control material.

REFERENCES

AABB Standards for Blood Banks and Transfusion Services, current edition

AABB Technical Methods, current edition

Fetal Hemoglobin Procedure 101, Rev. 11/2011, SURE-TECH Diagnostic Associates, Inc.

Paxton, Ann, "Bringing New Rigor to RhIG Calculations, CAP Today, May 2008

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
To update the test process to correspond to the latest revision of the package insert.			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☐ Date: ☒ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Kaaie Wilkinson, MD 12/17/14