

FETAL CELL STAIN KIT
Modified Kleihauer-Betke Acid Elution Procedure
For the Detection of Fetal Erythrocytes in Maternal Blood

PRINCIPLE & PURPOSE

This Kleihauer-Betke procedure, modified for room temperature testing, is based on the technique described by Kleihauer, et.al. in 1957. Their acid-elution procedure was the first to demonstrate the ability of fetal hemoglobin (HbF) to resist acid elution while adult hemoglobin (HbA) does not. This unique variation in staining, which leaves fetal erythrocytes (RBC's) a dark reddish-pink in color and adult RBC's a pale pink or white, allows one to easily distinguish between the fetal RBC's and adult RBC's in a peripheral blood smear.

This procedure is primarily used to determine the ratio of fetal cells to adult cells in a maternal blood sample collected post-delivery. This ratio is then used to estimate the volume of fetal-maternal hemorrhage (FMH) that occurred, and the amount of Rh Immune Globulin (RhIG) that is required to ensure the appropriate immune response in the (Rh) D-negative mother.

REAGENTS

All reagents in the Fetal Cell Stain Kit are stable when stored at room temperature for the interval indicated on each kit and/or reagent bottle. Expired reagents should be discarded, as required per laboratory safety guidelines.

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| <ol style="list-style-type: none"> 1. Reagents contained in the Fetal Cell Stain Kit: <ol style="list-style-type: none"> a. FETAL CELL FIXING SOLUTION (Ethanol, 80% w/v) b. FETAL CELL BUFFER SOLUTION (Citrate Buffer, 0.081M) c. FETAL CELL STAIN (Erythrosine-B, Fast Green) 2. All Fetal Cell Stain reagents are for in Vitro Diagnostic Use Only. | <ol style="list-style-type: none"> 3. All reagents should be handled using current laboratory safety guidelines. 4. Avoid contact with skin or eyes. 5. Fetal Cell Fixing Solution is flammable; avoid open flames while using. |
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EQUIPMENT & SUPPLIES

This procedure requires the following items not supplied in the Fetal Cell Stain kit:

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| <ol style="list-style-type: none"> 1. Microscope. 2. Glass slides 3. Immersion oil, as applicable | <ol style="list-style-type: none"> 4. Saline, 0.85% 5. Distilled water 6. Glass test tubes (12x75mm) | <ol style="list-style-type: none"> 7. Coplin jars (x4) 8. Timer |
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SPECIMEN COLLECTION & STORAGE

A maternal blood sample should be collected as soon as possible, post-delivery.

1. Specimen should be collected in EDTA
2. Sample may be stored at 2-8°C until tested.
3. For optimal results, testing should be performed within 24 hours of sample collection.

PROCEDURE

For optimal results, the following steps must be performed at room temperature, using room temperature reagents.

Prepare Control Samples:

1. Positive control samples may be prepared from a mixture of 1 part cord blood and 9 parts normal adult blood.
2. Negative control samples may be prepared using a blood sample from a normal adult male.
3. The control samples are then assayed in the same manner as the test samples, providing a qualitative confirmation of the technical procedure & reagents

Prepare Test & Control Smears:

1. Gently mix the sample:
 - a. Remove 2 drops of sample and place in a properly labeled glass test tube (12 x 75mm).
 - b. Add 3 drops of 0.85% Saline to the tube and mix by gentle agitation.
2. Place one drop of the diluted sample on one end of a properly labeled glass slide:
 - a. Prepare a thin smear by drawing the edge of another slide through the drop of blood and across the slide.
 - b. Repeat above steps for additional test and control samples, as needed.
3. Allow all slides to dry at room temperature before continuing with the staining process.
 - a. Once dried, the slides should be taken through the entire staining process as soon as possible.

NOTE: It is recommended that dried smears be viewed under 250x magnification to confirm a monolayer of cells. If not obtained, prepare new smears should be prepared.

Stain Slides:

1. Prepare Coplin jars:
 - a. Label 1 clean jar for each Fetal Cell Stain Kit reagent & 1 for distilled water.
 - b. Fill each jar with the appropriate reagent in sufficient volume to cover the smears.
2. Place the slides in vessel containing sufficient Fetal Cell Fixing Solution to cover the smears.
 - a. Allow the slides to remain in the solution for 5 minutes.
3. Remove the slides and rinse thoroughly in distilled water.
4. Allow slides to drain dry.
5. Place the slides in a clean vessel containing sufficient Fetal Cell Buffer Solution to cover the smears.
 - a. Allow slides to remain in the solution for 8-10 minutes.
6. Remove the slides from the buffer solution and immediately place in a clean vessel containing Fetal Cell Staining Solution.
 - a. Allow slides to remain in the staining solution for 3 minutes.
7. Remove the slides from the Fetal Cell Staining Solution and rinse thoroughly with distilled water.
8. Allow the slides to dry completely prior to microscopic review.

Microscopic Review of Slides:

1. Stained slides may be examined using high/dry or oil-immersion objective.
2. Fetal RBC's will stain a dark reddish-pink while normal adult RBC's will appear light pinkish-white with a slightly darker center.
3. Staining intensity of adult cells may vary slightly within and between lots of reagents; however, fetal and adult cells will be easily differentiated.
4. For optimal results, slides should be read within 24 hours of staining.

NOTE: Leukocytes (WBC's) may stain in varying degrees. WBC's may be distinguished from RBC's by the presence of a well-defined cell nucleus.

--See color slides below for assistance with distinguishing Fetal RBC's from Adult RBC's & WBC's:

Interpretation of Results:

1. Fetal Cells are typically reported as the ratio of fetal RBC's to adult RBC's, as determined by the counting number of each in randomly selected fields.
2. Record the number of fetal RBC's and the number of adult RBC's in each of 8-10 randomly selected monolayer fields.
3. Total the number of fetal RBC's counted in all fields and the number of adult RBC's counted in those fields.
4. Determine the ratio of fetal cells to adult cells by dividing the total number of fetal RBC's by the total number of adult RBC's:

EXAMPLE: Total # Fetal RBC's Counted = 26
 Total # Adult RBC's Counted = 4445
 Fetal RBC's/Adult RBC's Ratio = 26/4445 = 0.0058



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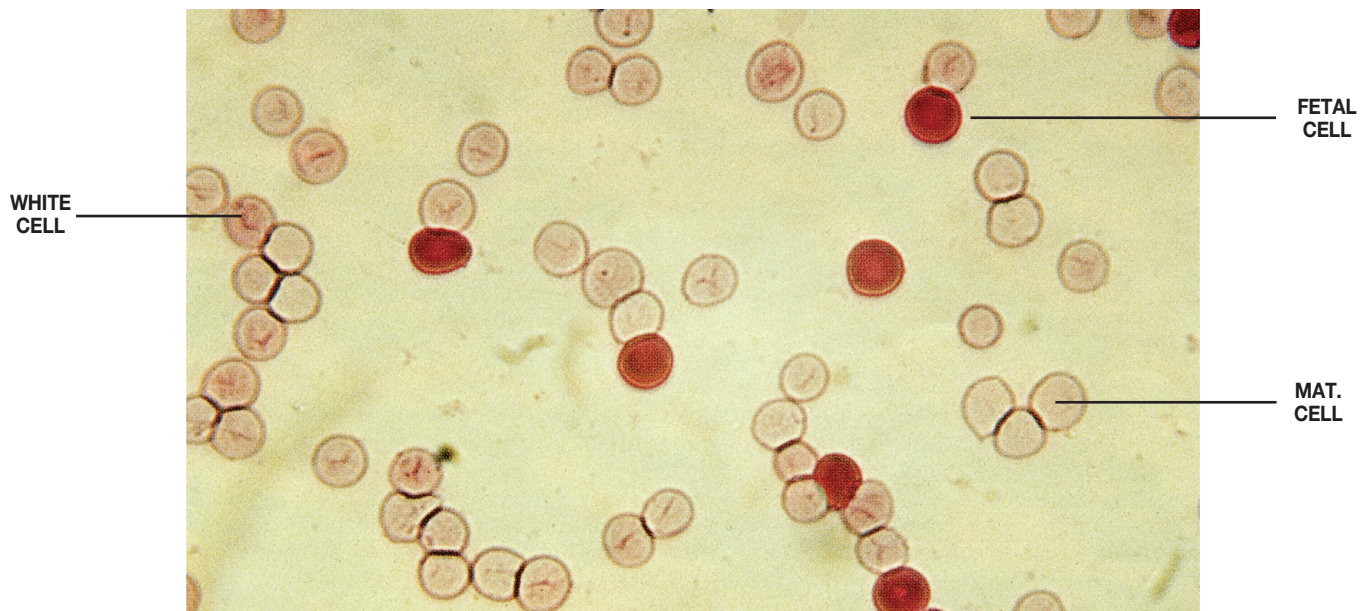
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Determination of RhIG Dosage:

1. The recommended number of vials of RhIG required to sufficiently protect against Rh immunization is determined using the fetal cell to adult cell ratio determined above.
2. In the left hand column of the chart below, locate the appropriate Fetal/Adult Cell range corresponding to the ratio calculated above.
3. Using the center column, determine the estimated volume of Fetal Maternal Hemorrhage (FMH) associated with that range of Fetal/Adult RBC ratio.
4. Using the right hand column, determine the recommended amount of RhIG (# of vials) required to cover the fetal-maternal hemorrhage (FMH).

Fetal/Adult RBC	Ratio Estimated FMH	Vials of RhIG Indicated
0 to 0.0045	up to 15 ml	1
0.0046 to 0.0090	from 15-30 ml	2
0.0091 to 0.0135	from 30-45 ml	3
0.0136 to 0.0180	from 45-60 ml	4
0.0181 to 0.0225	from 60-75 ml	5

NOTES:

- 1) If the Fetal/Adult RBC ratio exceeds the highest range in the first column (0.0225), the amount of RhIG indicated may be determined by adding one additional vial for each 0.0045 increase in the Fetal/Adult RBC ratio.
- 2) If the amount of RhIG indicated is less than a full vial, it is recommended that the full vial be administered.

****These are recommendations only; actual amount of RhIG to be administered should be reviewed & approved by the Primary Physician prior to administration****
PROCEDURAL NOTES & SOURCES OF ERROR

1. The number of slides to prepare per sample will vary with operator technique and experience.
2. The degree of elution of HbA may vary from patient to patient, therefore color variation among blood samples is to be expected.
3. Normal adult blood contains less than 1.0% of HbF.
 - a. Hematological disorders in adults may cause the production of abnormal levels of HbF.
 - b. Take care to select "normal" adult blood samples for use as controls.
4. Lymphocytes may take up stain in varying degrees, but typically less than fetal cells. WBC's can be distinguished from RBC's by their well-defined nucleus. (See illustrations in previous sections of this procedure).

QUALITY CONTROL & ASSURANCE

1. Current Regulatory & Accreditation Guidelines require a positive control sample be tested with each batch to confirm acceptable procedural performance.
2. CLIA '88 guidelines & Current Best Practice recommend both positive and negative controls be performed with each batch of special stains.
3. Preparation of both Positive & Negative controls are described in an earlier section of this procedure.
4. CLIA '88, as defined in 42CFR493, requires that all laboratories performing a Kleihauer-Betke staining procedure for HbF must verify the accuracy of their results at least twice yearly. The College of American Pathologists (CAP) Proficiency Tests are available for that purpose.

PRODUCT ORDER INFORMATION

Product #	Product Name	Product Size
S0412-00	FETAL CELL STAIN KIT	300 Tests
S0412-100	FETAL CELL STAIN KIT	100 Tests
S0411-06	CITRATE BUFFER SOLUTION	950ml
S0412C	FIXING SOLUTION	950ml
S0412A-100	STAINING SOLUTION	3X 120ml

KIT COMPONENTS

FETAL CELL FIXING SOLUTION (Ethanol, 80% w/v)
 FETAL CELL BUFFER SOLUTION (Citrate Buffer, 0.081M)
 FETAL CELL STAIN (Erythrosine-B, Fast Green)

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