Beaumont HEALTH

Beaumont Laboratory
Royal Oak

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Related Documents: NA

ACID ELUTION PROCEDURE
(FETAL HEMOGLOBIN IN RED BLOOD CELLS)
KLEIHAUER AND BETKE METHOD
SURE TECH REAGENTS

RC.HM.PR.036.r06

Principle

A citric acid-phosphate buffer solution 0.2 mol/L elutes or removes all hemoglobins except for Hemoglobin F from the red blood cells on an air-dried smear fixed in 80% ethanol. Only Hb F remains. The distribution is then determined after staining with Erythrosin 0.1%. Cells that stain with Erythrosin contain Hb F. Non-staining red cells do not contain Hb F. The percent of Erythrosin stained cells is calculated. This is an estimate of cells from the fetus that have entered the maternal circulation.

Specimen Collection and Handling

Type:	Whole blood collected in a vacutainer. This is the preferred sample. OR Capillary blood collected in a microtainer.	
Anticoagulant:	K_2EDTA	
Amount:	Whole blood	- Minimum sample size is 2.0 mL - Optimum sample size is 4.0 mL
	Capillary blood	- Minimum sample size is 250 mcL - Optimum sample size is 500 mcL
Special Handling:	Specimen must be well mixed for minimum of two minutes before being analyzed. *Trauma cases on* during off-shift hours *and weekends will be processed as soon as possible.* Any other specimens will be run the next working day by Flow Cytometry. In the event that a request is received from an internal source, i.e. Blood Bank, for the purpose of validating a positive screen, these requests will be performed as soon as possible by Hematology, without regard to original specimen status (stat, routine, etc.), patient location or reason why the test was initially ordered.	
Timing:	Specimen is stable for 2h at room temperature; 48h at 4^{o}C.	
Availability:	Trauma cases as soon as possible. (This is a backup method for Flow Cytometry Lab).	

Criteria for Unacceptable Specimens:	Specimens containing clots or insufficient volume are unacceptable and must be redrawn.

Supplies

Reagents

1. 0.85% Saline

2. **SURE- TECH Diagnostic, Associates, Inc. Fetal Hemoglobin Reagents,** to be stored at room temperature (20-26ºC); use until expiration date on label:

   a. **Red Cell Fixing Solution, Product No.101-10**
      80% (v/v) Ethyl Alcohol, denatured
   b. **Citrate/ \Phosphate Buffer, Product No. 101-20**
      0.2 mol/L
      Preservatives
   c. **Hemoglobin Staining Solution, Product No. 101-30**
      Erythrosin, 0.1%
      Stabilizers

   Reagents are provided ready to use. They are stored at room temperature and are stable for the period indicated on the label or until reagent turns cloudy or fetal erythrocytes are unable to be discerned on control slides.

Supplies

1. Staining Rack
2. Stain drying rack
3. Glass slides
4. Test tubes 12x75 mm
5. Microscope
6. Parafilm

Quality Control

Normal and abnormal control smears are run simultaneously with patient smears. A stock of frozen, fixed control slides is available for use. The slides are stored in the -70ºC freezer. Freshly prepared control slides may be used if the frozen stock is depleted or if they do not perform as expected.

The normal control should be prepared using a normal adult blood. It will show unstained (ghost cells) red blood cells. Identify slides as “Normal” and include order number of blood and date.

An abnormal control smear should show two distinct cell populations - one densely stained and one unstained (ghost cells). Prepare the abnormal control by mixing 5 drops of cord blood (cells off a clot are acceptable) and 5 drops from an ABO compatible specimen obtained from Blood Bank. This same ABO compatible specimen can be used when preparing the normal control. Prepare fresh on day of use, or use previously frozen, fixed control slides. Identify slides as “Abnormal” and include order number of cord blood and date.

To prepare a batch of control slides for freezing, see Attachment A.

Document stain quality in cytochemistry log book, along with results of the control slides.

Save control slides in "Special Stain Control Slides" file drawer for minimum of two years.

Procedure

1. Place 3 drops 0.85% saline and 2 drops of the abnormal control blood mixture into a 12x75 mm test tube, mix gently. Prepare 2 smears from this dilution and air dry at room temperature.

2. Repeat step 1 to prepare slides for the normal control blood and the patient blood.

3. Place the slides on a staining rack. Flood slides with sufficient Red Cell Fixing Solution to cover the smears. Allow the slides to remain covered with the solution at room temperature for **5 minutes**.

   NOTE: If using control slides from the frozen stock, it is not necessary to fix and rinse these slides. Remove parafilm and allow control slides to come to room temperature before proceeding to step 5.

4. Rinse slides thoroughly with deionized water and air dry.

5. Place the dry slides on a staining rack. Flood slides with sufficient Citrate/ Phosphate Buffer to cover the smears. Allow the slides to remain covered with solution at room temperature for **10 minutes**. Do not rinse.

6. Pour off buffer solution. Some samples cells may appear less refractile if the buffer is rinsed off with water. Do not rinse.

7. Place the wet slides on a staining rack. Flood slides with sufficient Hemoglobin Staining Solution to cover the smears. Allow the slides to remain covered with the stain at room temperature for **3 minutes**.

8. Rinse slides thoroughly with deionized water and allow to dry at room temperature.

9. Scan smears under 20X for fetal cells. Scan approximately 10 fields (or approximately 5000 RBC). Fetal cells will stain a dark reddish-pink with a smooth texture while adult cells will appear white to 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. Refer to the Sure-Tech Diagnostics Kleihauer-Betke Fetal Hemoglobin Training Manual for assistance in fetal cell identification.

10. If **three (3) or more** fetal cells are present, count the number of fetal cells at 100X among a minimum of 5000 RBC (count 555 RBC using Miller ocular):

    a. The Miller disk consists of a large square with a small square in one corner - the ratio of the large square area to small square area is 9:1 (see Figure A).

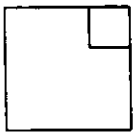

Figure A.

b. Use 100X oil objective.
c. Find appropriate counting area in thin portion of smear where cells are **evenly** distributed and not overlapping each other nor widely separated.
d. Count all **RBCs (mature and fetal) in the small square** and at the same time count the **fetal cells in the total large square**. Count consecutive fields until the RBCs counted equal 555.
e. Report the % RBCs that contain Hb F using calculation below.

11. If only an **occasional** fetal cell (1-2 per 5000 RBC) is present, report as "*less than 0.1%*".

Calculations and Interpretations

Using Miller ocular: $\frac{\text{\# fetal cells per 555 RBCs}}{50} = \%$ fetal cells

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Results

Red blood cells containing Hb F stain a dark reddish-pink with a smooth texture. RBCs containing Hb A, Hb S, or Hb C appear as ghosts, scarcely visible under oil immersion. Platelets will stain pink but are usually smaller with spike- like projections. Lymphocytes may stain pink but can be distinguished from fetal RBCs by their granular appearance caused by the nucleus. The outline of the nuclear membrane may also be visible.

Expected Results

Normal adult blood will contain less than 0.01% Hb F cells. The concentration of fetal hemoglobin, as a percentage of total, ranges from 64-95% at birth to approximately 5% at 6 months of age. Acceptable results for OB patients is less than 0.3%.

NOTE: 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.

NOTE: For those **rare** specimens greater than 1.0% (based on a review of WBH results over a 3 year period), request second sample and repeat to verify.

Reporting Results

In host computer, report results as:
(1) None seen
(2) Less than 0.1 %
(3) Actual %

Notes

1. On rare occasion, a request may be received for an acid elution stain on a bloody amniotic fluid.
   Proceed as follows:
   a) Make both cytocentrifuge smears **AND** smears from a cell button.
   b) Perform stain and count as usual.
2. Cord blood is fetal blood and is not suitable for assessing fetal/maternal hemorrhage. Cord blood is used for the preparation of abnormal control material.
3. Slides may be dried either by air-drying or the use of a small fan. The latter will improve turnaround times.

References

Miale JB. Laboratory medicine - hematology. St Louis: CV Mosby, 1982:885.

Fetal Hemoglobin product insert, SURE-TECH, Diagnostic Associates, Inc., St. Louis, MO

Kleihauer Betke Staining Procedure (Fetal Cell Stain). Document No. 7.1. Version 2. Revision Date: 11/2/2010.

Genesys Regional Medical Center Laboratory, Hematology Manual

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Attachments

Attachment A – FIXATIVE PROCEDURE FOR ACID ELUTION CONTROLS

Authorized Reviewers

Medical Director, Hematology

ACID ELUTION PROCEDURE (KLEIHAUER – BETKE) – SURE TECH REAGENTS

ATTACHMENT A – FIXATIVE PROCEDURE FOR ACID ELUTION CONTROLS

Specimen Obtain from Blood Bank:
1. Fresh cord blood
2. ABO compatible adult blood

Reagents
1. 0.85% Saline
2. **Red Cell Fixing Solution**, Product No. 101-10, SURE-TECH Diagnostic Associates, Inc., 80% (v/v) Ethyl Alcohol

Reagent is provided ready to use. Reagents are stored at room temperature and stable for the period indicated on the label or until reagent turns cloudy or fetal erythrocytes are unable to be discerned on control slides.

Supplies
Staining rack
Stain drying rack
Glass slides
Test tubes 12x75 mm
Pasteur pipettes
Microscope
Parafilm

Procedure

Abnormal Control Preparation:
1. Place 5 drops of cord blood and 5 drops of ABO compatible adult blood into a test tube. Mix gently.
2. Label a 12x75 mm test tube “Abnormal Control”
3. Place 3 drops 0.85% saline and 2 drops of the abnormal control blood mixture into the test tube. Mix gently.
4. Prepare 20 smears from this dilution. Label the slides “Abnormal” and include the order number of the cord blood and date. Air dry at room temperature for a minimum of 1 hour prior to fixation.

Normal Control Preparation:
1. Label a 12x75 mm test tube “Normal Control”.
2. Place 3 drops 0.85% saline and 2 drops ABO compatible adult blood in the tube. Mix gently.
3. Prepare 20 smears from this dilution. Label the slides “Normal” and include the order number of the ABO compatible adult blood and date. Air dry at room temperature for a minimum of 1 hour prior to fixation.

Place the slides on the staining rack. Flood slides with sufficient Red Cell Fixing Solution to cover the smears. Allow the slides to remain covered with the solution at room temperature for 5 minutes.

Rinse slides gently with deionized water and air dry. Allow to dry a minimum of 3 hours prior to storing in the freezer.

Wrap slides individually in parafilm. Place in all in a slide box in a designated area in the -70^{o}C freezer. Slides are stable for a minimum of 1 year.

ACID ELUTION PROCEDURE (KLEIHAUER – BETKE) – SURE TECH REAGENTS

Document Control

Location of Master: Hematology Procedure Manual
Master electronic file stored on the Clinical Pathology server:
S:\HEMACOAG\Document Control\Hematology\Procedure\Master Documents\Acid Elution.doc
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: E.Wystepek, MT(ASCP) M.Zamboldi H(ASCP)SH	03/2001			
Approved by: Joan C. Mattson, MD	03/14/2001			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Noelle Procopio, MT(ASCP)SH	12/30/2002		No change	
Noelle Procopio, MT(ASCP)SH	12/22/2003		No change	
Noelle Procopio, MT(ASCP)SH	12/15/2004		No change	
Joan C. Mattson, MD	02/17/2005	00	Standardized procedure format; updated K_3EDTA.	
Noelle Procopio, MT(ASCP)SH	10/24/2006		No change	
Ann Marie Blenc, MD	04/30/2007		No change; new director	
Ann Marie Blenc, MD	09/05/2007	01	Updated optimal sample size, pg. 1; removed references to Urgent testing, pg. 1&2; updated Analytical Cytometry to Flow Cytometry.	
Ann Marie Blenc, MD	03/13/2008		No change	
Ann Marie Blenc, MD	05/27/2009	02	Updated "EC" to "trauma cases"	
Ann Marie Blenc, MD	04/19/2010		No change	
Ann Marie Blenc, MD	04/20/2011		Updated procedure numbering only.	NA
Ann Marie Blenc, MD	09/11/2012	03	Modified quality control to utilize frozen slides; added Att. A (instructions for making control slides); updated terminology for SOFT.	NA
Ann Marie Blenc, MD	05/29/2014	04	Added Attachment section to body of procedure.	NA
Ann Marie Blenc, MD	04/16/2016		No change	NA
Ann Marie Blenc, MD	05/23/2017	05	Added directions on how to use the Miller ocular.	NA
Elizabeth Sykes, MD	02/02/2018			

Peter Millward, MD	01/30/2019		New Medical Director	
Ann Marie Blenc, MD	12/23/2019	**06**	Updated special handling and availability for trauma cases on off-shifts.	NA