**Fetal Hemoglobin Stain (Modified Kleihauer-Betke)**

**Purpose** This procedure provides instructions for performing the Fetal Hemoglobin Stain test.

**Background** The passage of erythrocytes from a Rh positive fetus into the circulation of a Rh negative mother results in the formation of specific Rh antibodies. In subsequent pregnancies, the Rh antibodies formed in the blood serum of the Rh negative mother are readily transmissible though the placenta into the circulation of the fetus. The action of the antibodies on the Rh postitive cells of the fetus may result in a disease entity recognized as isohemolytic desease, or erythroblastosis.

 The Modified Kleihauer-Betke procedure takes blood smears, which have been properly dried and fixed and immerses them in a citrate/phosphate buffer of pH 3.2. Adult hemoglobin (HbA) dissolves out of the cells, whereas fetal hemoglobin (HbF) which is acid resistant, remains intracellular and is stained and enumerated by microscopic examination in order to determine the amount of RhIg to administer to the mother in order to avoid formation of any Rh antibodies caused by any fetal bleed into the mother’s system which may have occurred.

**Specimen** Mother’s blood

* Anticoagulated (EDTA or oxalate)
* Stored at 2-8oC for up to 14 days.
* Specimens should be tested as soon as possible after collection.
* Hemolyzed specimens are not acceptable

**Materials**

|  |  |  |
| --- | --- | --- |
| Reagents | Supplies | Equipment |
| * Sure-Tech Fetal Hemoglobin Kit
* 0.9% Normal Saline
* DI Water
 | * Test tubes
* Test tube rack
* Disposable pipettes
* Glass slides
* Coplin jars
 | * Calibrated centrifuge
* Microscope
* Timer/Stopwatch
 |

**Quality**

**Control** Quality control slides must be run with each batch of slides stained.

* Positive Control slide-See procedure for preparation
* Negative Control slide-See procedure for preparation

**Procedure**

 **Quality Control Slide Preparation**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Obtain anticoagulated adult blood specimen that is antibody free. |
| 2 | Obtain a cord blood sample that is ABO compatible with the adult blood obtained in step 1. |
| 3 | Mix both samples thoroughly by inversion of the tubes. |
| 4 | Label one clean glass tube as the positive control |
| 5 | Label one clean glass tube as the negative control |
| 6 | Into the positive tube, place 10 drops of the adult blood specimen |
| 7 | Into the same glass tube, place 1 drop of the cord blood specimen |
| 8 | Mix the positive control by inversion or using a clean disposable pipette. |
| 9 | Into the negative control tube, place 11 drops of the same adult blood specimen obtained in step 1. |
| 10 | Assign control lot number by using the specimen numbers of the adult specimen and the cord blood specimen and combining them. See example.Ex. Adult specimen number is 0105:BB2 Cord specimen number is 0104:BB62 Lot number for Controls= 01052010462 |
| 11 | Proceed to staining procedure. |

 **Fetal Staining**

*NOTE: Staining Kit aliquot left in Coplin jars should only be used for 5 staining procedures. Using aliquot more times has been found to not allow for proper staining and has led to failure of QC.*

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Make sure that the temperature of the staining area is between 23oC and 27oC. Adjust temperature as necessary. |
| 2 | Mix the patient blood sample by gentle inversion. |
| 3 | Label clean test tubes for:1. Positive Control
2. Negative Control
3. Patient
 |
| 4 | Place 3 drops of saline into each tube |
| 5 | Place 2 drops of the corresponding blood into each labeled tube. |
| 6 | Mix tubes gently |
| 7 | Label one glass slide for each of the controls with the following information:1. Pos or Neg
2. Lot number
3. Date of testing
 |
| 8 | Label two glass slides for the patient, each with the following information:1. Patient full name
2. Patient MRN
3. Date of testing
 |
| 9 | Place one drop of the diluted blood on the corresponding glass slide near the end that is labeled. |
| 10 | Prepare a smear manually or using the slide maker. |
| 11 | Air dry the slide at room temperature. |
| 12 | If not already done, obtain the Fetal Stain Kit and fill Coplin jars with each of the solutions |
| 13 | Label each Coplin Jar with the OSHA label indicating the contents (See Appendix A: OSHA labeling of Coplin Jars for examples) |
| 14 | Place the slides in the Coplin jar containing the Red Cell Fixing Solution so that it covers the smear. |
| 15 | Raise and lower the slides 2-3 times for even distribution of the fixing solution. |
| 16 | Allow the slides to remain in the solution for 5 minutes |
| 17 | Remove the slides from the fixing solution and rinse thoroughly with DI water. |
| 18 | Allow to completely air dry |
| 19 | Place the slides in a Coplin jar containing Citrate/Phosphate Buffer ensuring that the solution is covering the smear. |
| 20 | Raise and lower the slides 2-3 times for even distribution of the buffer |
| 21 | Allow the slides to remain in the solution for 10 minutes |
| 22 | Remove the slides from the buffer solution and blot excess buffer from the slides |
| 23 | Place the wet slides in the Coplin jar containing Hemoglobin Staining Solution ensuring that the solution covers the smear. |
| 24 | Raise and lower the slides 2-3 times for even distribution of the Hemoglobin Staining Solution. |
| 25 | Allow the slides to remain in the solution for 3 minutes |
| 26 | Remove the slides from the Hemoglobin Stain Solution and rinse thoroughly with DI water |
| 27 | Allow to dry at room temperature. |

 **Examination of Slides**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Set up microscope so that slides can be viewed.* Condenser all the way down
* Brightness all the way up
* 40x dry magnification
 |
| 2 | Observe positive control for acceptability1. Adult cells appear white to light pink
2. Fetal cells appear dark reddish-pink
3. Fetal cells should be observed in at least every other observed field
 |
| 3 | Observe negative control for acceptability1. Adult cells appear white to light pink
2. No fetal cells observed
 |
| 4 | Count 1000 cells using the Miller Disc on one slide.* Start counting at the feathered edge of the stained smear.
* Count cells within the grid, being consistent regarding counting cells touching the grid lines.
 |
| 5 | Second tech will repeat the process from step 2, but will count the patient’s 2nd slide. |
| 6 | Refer to *Reporting Results* section for entry into the computer system/manual recording. |
| 7 | After reporting results in the LIS, place all slides in slide folder |
| 8 | Place slides and results report in bin for pathology review. |
| 9 | Place order sheet in stand-up file holder that is “Waiting for Path Review” |

**Interpretation**

*Patient can be Rh positive or negative in order for testing to be accurate.*

***NOTE: IF PATIENT IS RH POS, CONTINUE TO RESULTS SECTION AND THEN TO THE SEMI-QUANTITATIVE TESTING REPORTING.***

 **Determining Vials of RhIg to be Given**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Access the I:/ drive **on a computer terminal with Microsoft Excel** |
| 2 | Locate and double click on the *Blood Bank Excel Apps* Icon  |
| 3 | The RhIg Calculator spreadsheet will open. |
| 4 | Enter the patient’s full name, M# and the specimen number (ex. 0105:BB00001), into the designated fields in the calculator spreadsheet. |
| 5 | Enter the number of fetal cells counted by the first tech into the field for *Tech 1*. |
| 6 | Repeat step 5 for *Tech 2*. |
| 7 | The spreadsheet will automatically calculate the percent of the fetal bleed and the number of vials of RhIg to be given. |
| 8 | When all information is entered, click on the “File” tab in the top left corner of the screen. |
| 9 | Then click on the “Print” link on the left side of the screen |
| 10 | Make sure that the correct printer is chosen and then click the large *Print* button. |
| 11 | Attach the printout of the spreadsheet to the printed report sent to Pathology with the slides for review. |

**Result**

**Reporting**

**Reporting results in MCare**

* + - * + **Quantitative Test for Determination of RhIg Vial Dosage**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Log in to MCARE |
| 2 | Go to the “Specimen” module in the blood bank module. |
| 3 | Click on “Enter Results” |
| 4 | Type in Specimen M#, patient name or BB# to retrieve testing. |
| 5 | Select the specimen with the correct test to result |
| 6 | For the following fields, enter the corresponding results:--enter the lot number you assigned to the control slides--enter the initials of the tech who counted the first slide--enter “A” or “Acceptable” if pos control worked--enter “NP”-- enter “A” or “Acceptable” if neg control worked--enter number of fetal cells Tech 1 counted--enter “NP”--enter the initials of the tech who counted the second slide--enter “A” or “Acceptable” if pos control worked--enter “NP”-- enter “A” or “Acceptable” if neg control worked--enter the number of fetal cells Tech 2 counted--enter “NP”--enter the percentage shown on the spreadsheet --enter the number of vials of RhIg shown on the spreadsheet |
| 7 | After you entered all the required data, click  at the bottom of the screen or you can press F12 on the keyboard to verify the results. |
| 8 | The following screen will appear. Make sure that *Print External Inquiry after Filing* is checked by clicking on it. Then press F12 or click **Save** |
| 9 | The following screen will then appear. If the correct printer is not already chosen, go to the *ALL PRINTERS* and click on the “+” sign shown below to find your local printer |
| 10 | Click on that printer and then press F12 or click **OK** to save and print. |
| 11 | Attach print out of results and the printout from the RhIg Dose Calculator together and send with slides to pathology for review. |

**Reporting results on manual testing form.**

|  |  |
| --- | --- |
|  | **Action** |
| 1 | Attach a Meditech label or manually write the patient name, M# and DOB at the top of the Patient Manual Testing Worksheet. |
| 2 | In the Kleihaur-Betke Stain section of the worksheet, record the following from your observations for each tech:1. Pos QC was acceptable or not by recording “ACC” in the *High Pos* field2. Neg QC was acceptable or not by recording “ACC” in the *Normal Pos* field3. Number of fetal cells counted out of 1000 cells |
| 3 | Follow the directions in the above section **Determining Vials of RhIg to be Given.** |
| 4 | Record the *Reported % Fetal* and the *Vial Rhogam* from the RhIg Dose Calculator print out on the Worksheet. |
| 5 | Attach the Patient Manual Testing worksheet and the RhIg Dose Calculator printouts together and submit to pathology with the slides for review. |

**Reporting results if *RhIg Dose Calculator* is down.**

|  |  |
| --- | --- |
|  | **Action** |
| 1 | Attach a Meditech label or manually write the patient name, M# and DOB at the top of the Patient Manual Testing Worksheet. |
| 2 | In the Kleihaur-Betke Stain section of the worksheet, record the following from your observations for each tech:1. Pos QC was acceptable or not by recording “ACC” in the *High Pos* field2. Neg QC was acceptable or not by recording “ACC” in the *Normal Pos* field3. Number of fetal cells counted out of 1000 cells |
| 3 | Calculate the volume of the bleed:

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Divide the number of fetal cells counted by Tech 1 by 1000 (do not round) |
| 2 | Divide the number of fetal cells counted by Tech 2 by 1000 (do not round) |
| 3 | Add the result from step 1 to the result from step 2 (do not round) |
| 4 | Divide the result from step 3 by 2 (do not round) |
| 5 | Multiply the result from step 4 by 5000 (do not round) |
| 6 | The result is the volume of the bleed |

**Ex.** Tech 1 counted 5 cells Tech 2 counted 6 cells5/1000=0.005 6/1000=0.006(0.005+0.006)/2=0.00550.0055\*5000=27.50 mLs |
| 4 | Calculate the percent fetal blood:

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Divide the volume of the bleed by 5000(do not round) |
| 2 | Multiply the result from step 1 by 100 |
| 3 | The result is the percent fetal blood |

**Ex.** 27.50 mLs/5000=0.00550.0055\*100=0.55% |
| 5 | Calculate the recommended number of RhIg vials:

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Divide the volume of the bleed by 30(do not round) |
| 2 | Round the result from step 1 to the whole number* If the number after the decimal point is <5, round down
* If the number after the decimal point is ≥ 5, round up.
 |
| 3 | Add 1 to the results from step 2 |
| 4 | The result is the recommended number of RhIg vials. |

**Ex.** 27.50 mLs/30=0.91666666670.9166666667 rounds to 11+1= 2 vials of RhIg |
| 6 | Record the *Reported % Fetal, Volume of Bleed* and the *Vial Rhogam* from the calculations on the worksheet. |
| 7 | Submit to pathology, the Patient Manual Testing worksheet and the slides for review. |

* + - * + **Reporting for Semi-Quantitative Test (Rh Pos Individuals)**

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Log in to MCARE |
| 2 | Go to the “Specimen” module in the blood bank module. |
| 3 | Click on “Enter Results” |
| 4 | Type in Specimen M#, patient name or BB# to retrieve testing. |
| 5 | Select the specimen with the correct test to result |
| 6 | For the following fields, enter the corresponding results (RhIg Dosage Calulator is not used):--enter the lot number you assigned to the control slides--enter the initials of the tech who counted the first slide--enter “A” or “Acceptable” if pos control worked--enter “NP”-- enter “A” or “Acceptable” if neg control worked--enter number of fetal cells Tech 1 counted--enter “1000”--enter the initials of the tech who counted the second slide--enter “A” or “Acceptable” if pos control worked--enter “NP”-- enter “A” or “Acceptable” if neg control worked--enter the number of fetal cells Tech 2 counted--enter “1000”--MCare will calculate -- MCare will calculate |
| 7 | The following comment will attach to the results. |
| 7 | After you entered all the required data, click  at the bottom of the screen or you can press F12 on the keyboard to verify the results. |
| 8 | The following screen will appear. Make sure that *Print External Inquiry after Filing* is checked by clicking on it. Then press F12 or click **Save** |
| 9 | The following screen will then appear. If the correct printer is not already chosen, go to the *ALL PRINTERS* and click on the “+” sign shown below to find your local printer |
| 10 | Click on that printer and then press F12 or click **OK** to save and print. |
| 11 | Attach print out of results and send with slides to pathology for review. |

**References** AABB. *Standards for Blood Banks and Transfusion Services*--*29th Edition*. Std. 5.30.2, 5.30.3, 5.30.5. Bethesda, MD: American Association of Blood Banks; 2014

AABB. METHOD 5-2. TESTING FOR FETOMATERNAL HEMORRHAGE—MODIFIED KLEIHAUER-BETKE TEST**.** In: AABB. *Technical Manual*--*18th Edition*. Bethesda, MD: AABB; 2014.

Sure-Tech Diagnostic Associates Inc., *Fetal Hemoglobin stain package insert*, St. Louis, MO, Rev. 11/11.

**Related**

**Documents**

Appendix A: OSHA labeling of Coplin Jars

Appendix B: Fetal Hemoglobin Stain Flowchart

**.**

**Appendix A: OSHA Labeling of Coplin Jars**

Labels attached to sheet 

**PROCEDURE AND FORM CHANGE CONTROL**

|  |
| --- |
| Title: Fetal Hemoglobin Stain |
| Written | **Validated** | **Path Review** | **Review** | **Effective** | **Reason for Revision** |
| Date | **By** | **Date** | **By** | **Date** | **By** | **Date** | **By** | **Date** | **By** |
| **11/10/09** | **PAB** | **11/12/09** | **GJM** | **11/02/09** | **JAP** |  |  | **12/01/09** | **PAB** |  |
| **Revised** |  |  |  |  |  |  |  |  |  |  |
| **09/02/10** | **GJM** |  |  |  |  | **9/9/10** | **PAB** | **9/9/10** | **PAB** | **DI water from tap in department** |
|  |  |  |  |  |  | **3/29/11** | **PAB** |  |  |  |
| **6/28/11** | **PAB** |  |  | **7/5/11** | **ESB** |  |  | **7/6/11** | **PAB** | **Computer entry of results** |
|  |  |  |  |  |  | **7/18/12** | **PAB** |  |  |  |
| **10/26/12** | **PAB** |  |  | **11/16/12** | **ESB** |  |  | **1/1/13** | **PAB** | **Removal of premade controls** |
| **1/11/13** | **PAB** |  |  |  |  |  |  | **1/11/13** | **PAB** | **Added control storage and prep** |
| **2/5/13** | **PAB** | **2/6/13** | **LJA** | **2/8/13** | **ESB** |  |  | **2/14/13** | **PAB** | **Changed reagent storage temp, expiration time, removal of 1 rinse step.** |
| **2/10/14** | **PAB** |  |  | **2/11/14** | **ESB** |  |  | **2/14/14** | **PAB** | **Removed R# from labeling** |
| **12/17/15** | **JLH** |  |  |  |  |  |  | **12/17/15** | **JLH** | **New header and BB#** |
| **1/5/16** | **JLH** |  |  | **1/18/16** | **ESB** |  |  | **1/21/16** | **JLH** | **Change in control use, added instructions for entry, added RhIg dose calculator and more detail** |
| **1/22/16** | **JLH** |  |  | **1/22/16** | **ESB** |  |  | **1/26/16** | **JLH** | **Added downtime calculation** |
|  |  |  |  |  |  |  |  |  |  |  |

Location of any copy(s) of the procedure:

**Out of use:**

Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_By:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Reason:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_