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Last Approved Revision Date:	10/2016
Responsible Department:	Laboratory
Responsible Person:	Laboratory Medical Director

Signature:

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 through the placenta into the circulation of the fetus. The action of the antibodies on the Rh positive cells of the fetus may result in a disease entity recognized as iso-hemolytic disease, 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 2-8°C 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 1. Test tube rack 2. Disposable pipettes 3. Glass slides 4. Coplin jars	• Calibrated centrifuge • Microscope • Timer/Stopwatch

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

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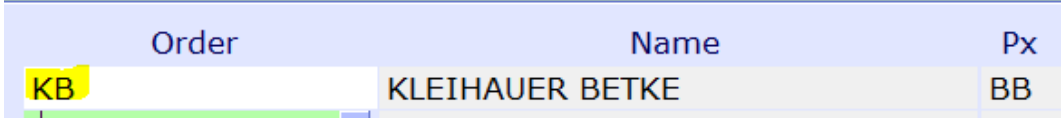
- Positive Control slide-See procedure for preparation
- Negative Control slide-See procedure for preparation

Procedure

Contents

1. Test Ordering
2. Determination of Test Flow
3. Computer Entry
4. Quality Control Slide Preparation
5. Fetal Cell Staining
6. Examination of Slides

1. Test Ordering

Step	Action
1	Nursing should order Kleihauer-Betke testing on the OM side.
2	If ordered on the lab side of MCARE, order KB in the Enter/Edit tab of the Specimen tab. 

2. Determination of Test Flow

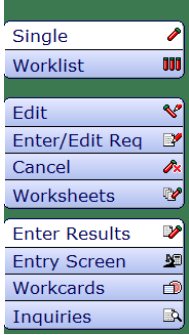
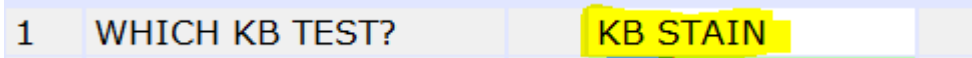
Step	Action				
1	Tech must determine if the fetal hemoglobin stain will be performed or if the testing will be sent to Barnes-Jewish hospital for flow cytometry testing. • Note: all efforts should be made to send testing for flow as this is the better testing and technique for fetal hemoglobin determination.				
2	Refer to the chart below.				
3	<table><tr><th>Send for Flow Cytometry if any of the following...</th><th>Perform FHS if any of the following....</th></tr><tr><td> • M-F between 0600-1900 • Routine • For determination of RhIg to be given after normal delivery • Requested by physician </td><td> • Weekends or holidays • After 1900 and before 0600 M-F • Doctor determines it cannot wait • It is a weekend or holiday and patient will be discharged before next regular run of flow. </td></tr></table>	Send for Flow Cytometry if any of the following...	Perform FHS if any of the following....	• M-F between 0600-1900 • Routine • For determination of RhIg to be given after normal delivery • Requested by physician	• Weekends or holidays • After 1900 and before 0600 M-F • Doctor determines it cannot wait • It is a weekend or holiday and patient will be discharged before next regular run of flow.
Send for Flow Cytometry if any of the following...	Perform FHS if any of the following....				
• M-F between 0600-1900 • Routine • For determination of RhIg to be given after normal delivery • Requested by physician	• Weekends or holidays • After 1900 and before 0600 M-F • Doctor determines it cannot wait • It is a weekend or holiday and patient will be discharged before next regular run of flow.				
4	If it is determined that the testing will be performed by flow cytometry, then refer to the <i>Fetal Hemoglobin by Flow Cytometry</i> procedure.				

3. Computer Entry

Step	Action
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1	Log in to MCARE				
2	Go to the Specimen tab 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	Enter S for KB STAIN in the Which Test? field, or press F9 for options. 				
7	The next field will appear which is Previous BT? Enter Y if the patient has a historical blood type or N if the patient does not have a historical blood type.				
8	<table border="1"> <thead> <tr> <th>If you entered "N"...</th><th>If you entered "Y"....</th></tr> </thead> <tbody> <tr> <td> - The system will order an ABORH - Perform the ABORH and enter the results before proceeding. - See appropriate SOP for directions on performing and resulting ABORH. - Proceed to step 9. </td><td> - Proceed to step 9. </td></tr> </tbody> </table>	If you entered "N"...	If you entered "Y"....	- The system will order an ABORH - Perform the ABORH and enter the results before proceeding. - See appropriate SOP for directions on performing and resulting ABORH. - Proceed to step 9.	Proceed to step 9.
If you entered "N"...	If you entered "Y"....				
- The system will order an ABORH - Perform the ABORH and enter the results before proceeding. - See appropriate SOP for directions on performing and resulting ABORH. - Proceed to step 9.	Proceed to step 9.				
9	In the PAT'S RH TYPE? field, enter the patient's Rh type from the current ABORH or historical ABORH. N for negative P for positive				
10	Proceed to the next section for the performance of the stain.				

4. 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

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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.

5. Fetal Cell 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 23°C and 27°C. 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:

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	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.

5. 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 acceptability 1. 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 acceptability 1. 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 2 nd slide.
6	Refer to <i>Reporting Results</i> section for entry into the computer system/manual recording.
7	After reporting results in the LIS, place all slides in slide folder

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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.

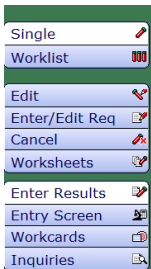
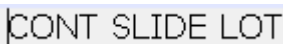
Result Reporting

Reporting results in MCare

Contents

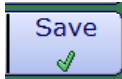
1. Quantitative Test for Determination of RhIg Vial Dosage
2. Reporting results on manual testing form.
3. Reporting for Semi-Quantitative Test (Rh Pos Individuals)

1. 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

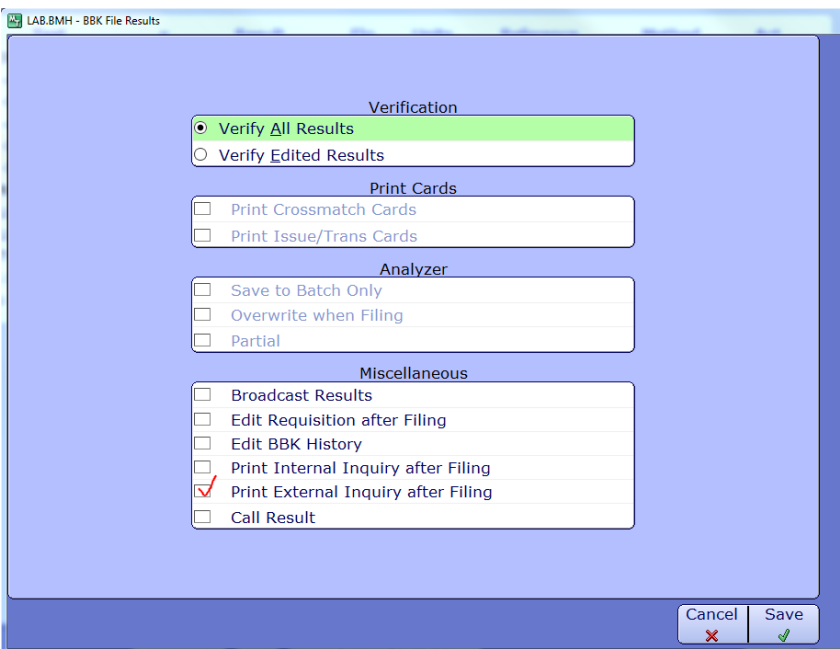
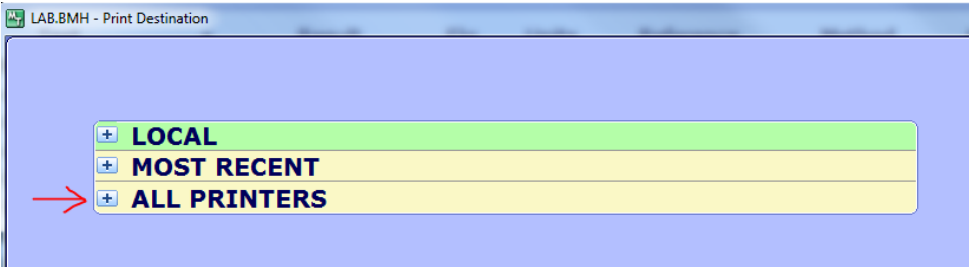
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	TECH1 INITIALS --enter the initials of the tech who counted the first slide STRONG POS CTRL --enter "A" or "Acceptable" if pos control worked WEAK POS CTRL --enter "NP" NEG CTRL -- enter "A" or "Acceptable" if neg control worked T1 FETAL CELLS --enter number of fetal cells Tech 1 counted T1 TOTAL CELLS --enter 1000 TECH2 INITIALS --enter the initials of the tech who counted the second slide STRONG POS CTRL --enter "A" or "Acceptable" if pos control worked WEAK POS CTRL --enter "NP" NEG CTRL -- enter "A" or "Acceptable" if neg control worked T2 FETAL CELLS --enter the number of fetal cells Tech 2 counted T2 TOTAL CELLS --enter 1000 The rest will automatically calculate.
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 comment will attach. <pre> ----- VIALS OF RHIG 7 H 0-1 BMH %Acid Resistant Cells Vials RhIg to Inject 0.0-0.2 1 0.3-0.8 2 0.9-1.4 3 1.5-2.0 4 2.1-2.6 5 >2.6 Calculated Note: THESE ARE PRELIMINARY RESULTS. FINAL RESULTS SUBJECT TO CHANGE PENDING PATHOLOGIST REVIEW </pre>
8	The following screen will appear. Make sure that <i>Print External Inquiry after Filing</i> is checked by clicking on it. Then press F12 or click Save

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9	The following screen will then appear. If the correct printer is not already chosen, go to the <i>ALL PRINTERS</i> 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.

2. Reporting results on manual testing form.

	Action
1	Attach a Mediatech 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 <i>High Pos</i> field 2. Neg QC was acceptable or not by recording “ACC” in the <i>Normal Pos</i> field 3. Number of fetal cells counted out of 1000 cells
3	Calculate the volume of the bleed:

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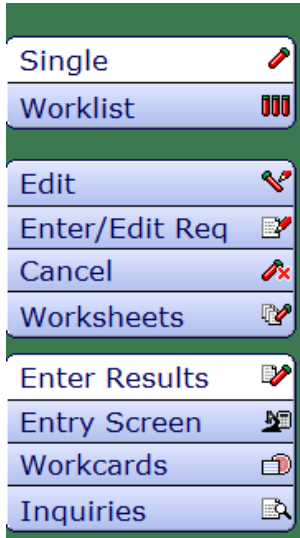
	Step	Action
	1	Divide the number of fetal cells counted by Tech 1 by 1000 (do not round)
	2	Divide the number of fetal cells counted by Tech 2 by 1000 (do not round)
	3	Add the result from step 1 to the result from step 2 (do not round)
	4	Divide the result from step 3 by 2 (do not round)
	5	Multiply 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 cells $5/1000=0.005$ $6/1000=0.006$ $(0.005+0.006)/2=0.0055$ $0.0055*5000=27.50 \text{ mLs}$		
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. $27.50 \text{ mLs}/5000=0.0055$ $0.0055*100=0.55\%$		
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. $27.50 \text{ mLs}/30=0.916666667$ 0.916666667 rounds to 1 $1+1= 2 \text{ vials of RhIg}$		
6	Record the <i>Reported % Fetal</i> , <i>Volume of Bleed</i> and the <i>Vial Rhogam</i> from the calculations on the worksheet in MCARE in the appropriate fields.	

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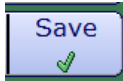
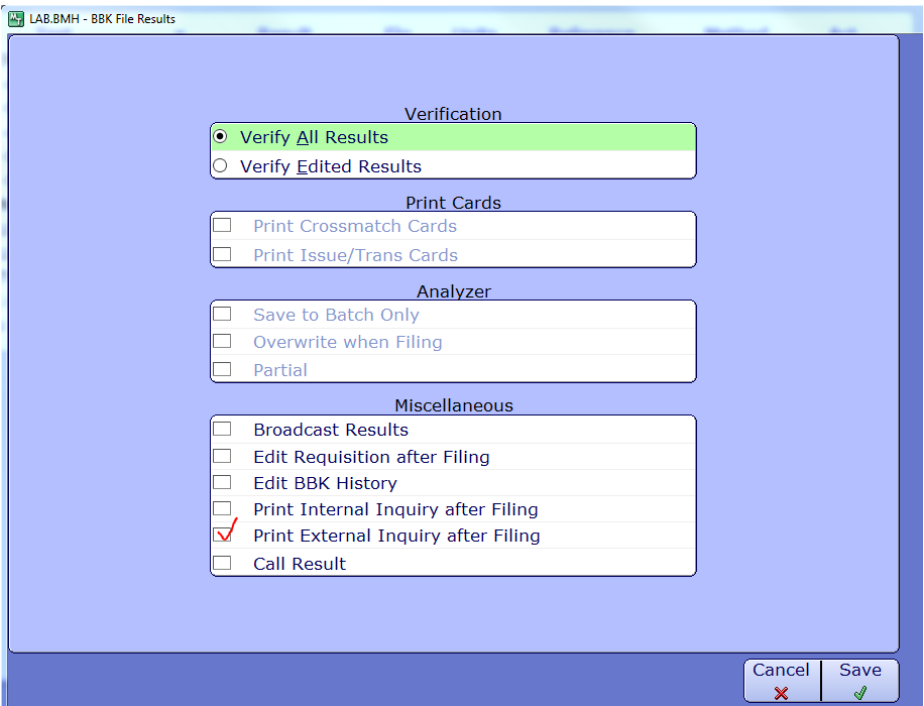
7	Submit to pathology, the Patient Manual Testing worksheet and the slides for review.
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3. Reporting for Semi-Quantitative Test (Rh Pos Individuals)

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 CONT SLIDE LOT --enter the lot number you assigned to the control slides TECH1 INITIALS --enter the initials of the tech who counted the first slide STRONG POS CTRL --enter "A" or "Acceptable" if pos control worked WEAK POS CTRL --enter "NP" NEG CTRL -- enter "A" or "Acceptable" if neg control worked T1 FETAL CELLS --enter number of fetal cells Tech 1 counted T1 TOTAL CELLS --enter "1000" TECH2 INITIALS --enter the initials of the tech who counted the second slide STRONG POS CTRL --enter "A" or "Acceptable" if pos control worked

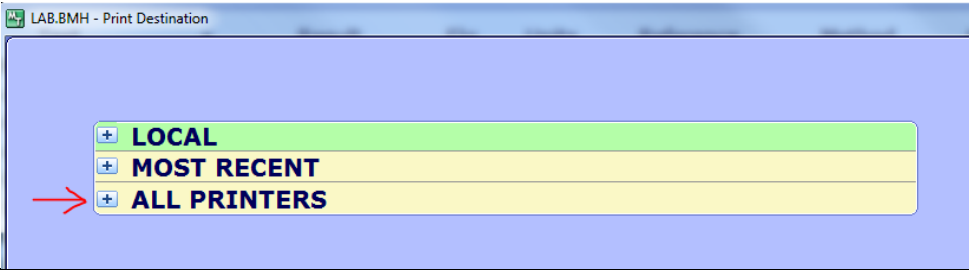
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	WEAK POS CTRL --enter "NP" NEG CTRL -- enter "A" or "Acceptable" if neg control worked T2 FETAL CELLS --enter the number of fetal cells Tech 2 counted T2 TOTAL CELLS --enter "1000" PCT ACID RES --MCare will calculate FETAL BLEED -- MCare will calculate										
7	The following comment will attach to the results. <table border="1"> <thead> <tr> <th>% Acid Resistant Cells</th> <th>Semi-Quantitative Results</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>None Seen</td> </tr> <tr> <td>0.1-0.8</td> <td>Small</td> </tr> <tr> <td>0.9-1.4</td> <td>Moderate</td> </tr> <tr> <td>1.5 or Greater</td> <td>Large</td> </tr> </tbody> </table> <i>Note: THESE ARE PRELIMINARY RESULTS. FINAL RESULTS SUBJECT TO CHANGE PENDING PATHOLOGIST REVIEW.</i>	% Acid Resistant Cells	Semi-Quantitative Results	0.0	None Seen	0.1-0.8	Small	0.9-1.4	Moderate	1.5 or Greater	Large
% Acid Resistant Cells	Semi-Quantitative Results										
0.0	None Seen										
0.1-0.8	Small										
0.9-1.4	Moderate										
1.5 or Greater	Large										
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 <i>Print External Inquiry after Filing</i> 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 <i>ALL PRINTERS</i> and click on the "+" sign shown below to find your local printer										

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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

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Appendix A: OSHA Labeling of Coplin Jars

Red Cell Fixing Sol'n
CHEMICAL NAME
##/####
SDS# expiration

HAZARD KEY
4 - SEVERE
3 - SERIOUS
2 - MODERATE
1 - SLIGHT
0 - MINIMAL

☒ 3 FIRE HAZARD
☒ 2 HEALTH HAZARD
☐ 0 INSTABILITY
☐ 0 PERSONAL PROTECTION

PERSONAL PROTECTION KEY

A	SP2	G	SP2 + M2 + *
B	SP2 + M2	H	SP2 + M2 + * + *
C	SP2 + M2 + *	I	SP2 + M2 + *
D	SP2 + M2 + *	J	SP2 + M2 + * + *
E	SP2 + M2 + *	K	SP2 + M2 + *
F	SP2 + M2 + *	X	See your supervisor for specialized handling directions

MANUFACTURER: Sure-Tech
PHONE: 314-894-8932

HbO Staining Sol'n
CHEMICAL NAME
##/####
SDS# expiration

HAZARD KEY
4 - SEVERE
3 - SERIOUS
2 - MODERATE
1 - SLIGHT
0 - MINIMAL

☐ 0 FIRE HAZARD
☒ 2 HEALTH HAZARD
☐ 0 INSTABILITY
☐ 0 PERSONAL PROTECTION

PERSONAL PROTECTION KEY

A	SP2	G	SP2 + M2 + *
B	SP2 + M2	H	SP2 + M2 + * + *
C	SP2 + M2 + *	I	SP2 + M2 + *
D	SP2 + M2 + *	J	SP2 + M2 + * + *
E	SP2 + M2 + *	K	SP2 + M2 + *
F	SP2 + M2 + *	X	See your supervisor for specialized handling directions

MANUFACTURER: Sure-Tech
PHONE: 314-894-8933

Citrate/Phosphate Buffer
CHEMICAL NAME
##/####
SDS# expiration

HAZARD KEY
4 - SEVERE
3 - SERIOUS
2 - MODERATE
1 - SLIGHT
0 - MINIMAL

☐ 0 FIRE HAZARD
☒ 2 HEALTH HAZARD
☐ 0 INSTABILITY
☐ 0 PERSONAL PROTECTION

PERSONAL PROTECTION KEY

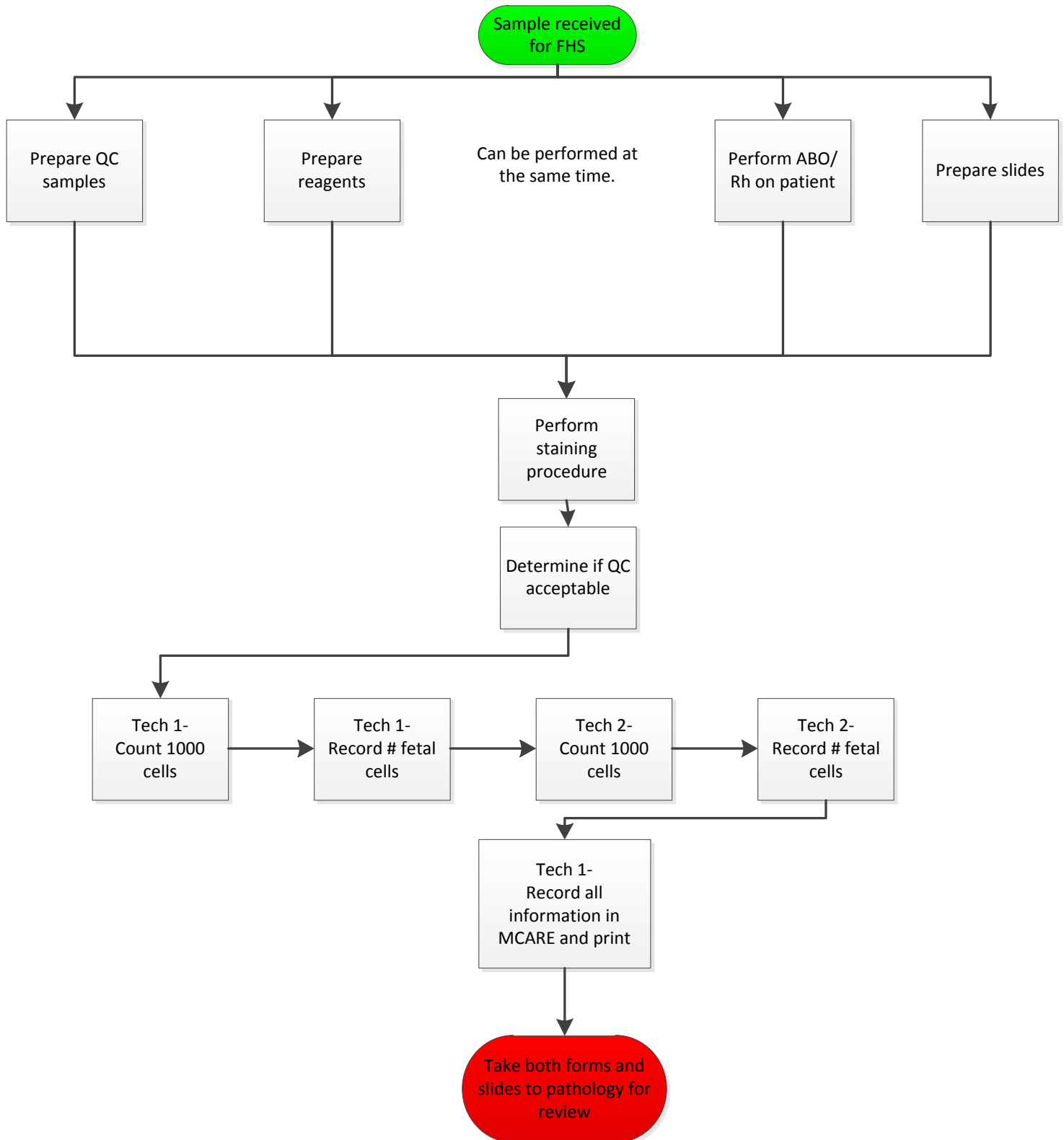
A	SP2	G	SP2 + M2 + *
B	SP2 + M2	H	SP2 + M2 + * + *
C	SP2 + M2 + *	I	SP2 + M2 + *
D	SP2 + M2 + *	J	SP2 + M2 + * + *
E	SP2 + M2 + *	K	SP2 + M2 + *
F	SP2 + M2 + *	X	See your supervisor for specialized handling directions

MANUFACTURER: Sure-Tech
PHONE: 314-894-8933

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Appendix B: Fetal Hemoglobin Stain Flowchart



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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

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										calculation
2/9/16	JLH							2/9/16	JLH	New header
10/10/16	JLH	10/5/16	GJM	10/14/16	ESB			10/21/16	JLH	Changed to accommodate sending some specimens for flow cytometry.

Location of any copy(s) of the procedure:

Out of use:

Date:_____By:_____Reason:_____