TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	5.30.2014
Department:	Blood Bank	**Due Date:**	6.8.2014

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Fetal Bleed Screening

Description of change(s):

1. Fetal screens are only performed at SGAH (no longer at WAH).
2. SGAH staff members must fax WAH results to the WAH pharmacy.
3. Removed instructions for ordering the KBT and refer to the reader to the procedure, "Order Entry, Entering Orders in the GUI System."
4. Other wording was updated to provide more clarity.

Technical SOP

Title	**Fetal Bleed Screening**	
Prepared by	Stephanie Codina	Date: 11/14/2012
Owner	Stephanie Codina	Date: 11/14/2012

Laboratory Approval	**Local Effective Date:**	
Print Name	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Fetal Bleed Screening	Manual	FSCR, RHOG

Synonyms/Abbreviations
FetalScreen, RhIG Evaluation

Department
Blood Bank

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

A red blood cell suspension from a D-negative mother is incubated with reagent containing anti-D and then washed to remove unbound antibody. A weak suspension of D-positive red blood cells is added. The red blood cell mixture is centrifuged and examined microscopically for mixed-field agglutination. Since any minor population of D-positive red cells will have become coated with anti-D during the incubation phase, the D-positive indicator cells added after washing form rosettes around the individual cells of the minor population, leading to larger and readily detected agglutinates. In most cases the fetomaternal hemorrahage is not sufficient to cause a positive test, but in those cases where a significant volume of fetal blood has entered the maternal circulation, the test provides an indication that a quantitative test is required to determine whether the bleed was sufficient to warrant a larger dose of RhIG to the mother.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Specimen Collection and/or Timing	Maternal specimen collected after delivery of all products of conception. It is best to wait approximately 1 hour after delivery to allow fetal cells to mix with maternal circulation if present.
Other	Testing is not required if patient is less than 20 weeks gestation.

3.2 Specimen Type & Handling

Criteria		
Labeling	Patient identification must be confirmed. Refer to procedure 'Sample Specifications for Blood Bank Testing' for details.	
Type -Preferred **-Other Acceptable**	Plasma (EDTA) None	
Collection Container	Lavender top tube	
Volume - Optimum **- Minimum**	3ml 2ml	
Transport Container and Temperature	Same as above, at room temperature	
Stability & Storage Requirements	Room Temperature:	N/A, testing performed ASAP
	Refrigerated:	2 days
	Frozen:	Unacceptable
Timing Considerations	Testing performed ASAP to permit administration of RhIG within 72 hours of delivery.	

Criteria	
Unacceptable Specimens & Actions to Take	Frozen, Incomplete or incorrect labeling – refer to procedure 'Sample Specifications for Blood Bank Testing' for details.
Compromising Physical Characteristics	Do not use grossly hemolyzed samples for testing. Bacterial contamination may cause false positive results.
Other Considerations	None

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
FMH RapidScreen	Immucor 7893 or equivalent
pHix Buffer Solution	Immucor 5070 or equivalent
Isotonic Saline, Certified blood bank saline	Fisher 23535435 or equivalent

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Assay Kit - FMH RapidScreen	
Reagent a	Anti-D, monoclonal, IgM, GAMA401
Reagent b	Indicator Cells
Reagent c	Positive Control
Reagent d	Negative Control
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Gently resuspend cell suspensions before using.

Reagent	pHix Buffer Solution
Preparation and Storage/Stability	Add 1 bottle (200 ml.) pHix to one cube of isotonic saline (20L). Store at ambient temperature (18-30°C) for a maximum of 30 days. pH of buffered saline should be 6.5 – 7.5 as tested with a pH strip. Record lot#, expiration date and pH on Phix Log.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Positive and Negative Controls supplied with kit.

6.2 Control Preparation and Storage

Refer to section 4.2.

6.3 Frequency

Both controls are run in parallel with each batch of tests (or with each test if performed singly).

6.4 Tolerance Limits

Expected results:
Positive control must have 5 or more agglutinants per five low-power fields
Negative control must have 4 or fewer agglutinants per five low-power fields

For the patient test result to be considered valid, the result of the negative control cell run at the same time must be negative and the result obtained with the positive control cells run at the same time must be positive. The test is invalid if either control yields unacceptable results and testing must be repeated. Do not report results and notify and supervisor if controls fail a second time.

6.5 Review Patient Data

Review patient results for unusual patterns, trends or distributions in patient results, such as an unusually high percentage of abnormal results, or unusually high percentage of non-reactive, or indeterminate, or reactive results. Computer aided tools should be used when available.

6.6 Documentation

Results are recorded on appropriate worksheet.

6.7 Quality Assurance Program

Participation in CAP proficiency testing

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

- Timer
- Centrifuge
- Microscope

7.2 Supplies

- Test tubes, 12 x 75 mm
- Disposable pipettes
- Microscope slides
- Phosphate Buffered Saline, 0.9%, pH 6.5 – 7.5

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included in the appropriate notebook/file. The components of each kit should be used together.

Step	Action
1	Verify the history of both the mother and the infant. The following must apply, A. The mother must have a current T&S to ensure she is Rh-negative and does not have anti-D. Request a T&S order if she does not have a current specimen on file. B. The mother must be Rh-negative. C. The infant must be Rh-positive. Blood bank staff members will cancel the fetal screen and order a Kleihauer-Betke test in the following situations: A. Infant is weak D positive (false negative results may occur). B. Mother is weak D positive (false positive results may occur). C. Fetal/infant blood type is unknown or Rh-negative (results of the fetal screen test may be invalid). **Note:** SGAH - The physician or patient care area will order the fetal cell screen testing. WAH - Blood bank staff members will order fetal cell screen or Kleihauer-Betke testing on any Rh-negative or weak D negative mother that delivers an Rh-positive or weak D positive infant based on cord blood evaluation testing.
2	Label 4 test tubes: 1. Label 2 tubes with the patient identifiers (one tube is for the cell suspension and one tube is for testing). At a minimum, the tubes should be labeled with the patient's first and last initial or the first 3 letters of the patient's last name. 2. Label 1 tube "POS" for the positive control. 3. Label 1 tube "NEG" for the negative control.
3	Prepare a 2-4 % suspension of the maternal red blood cells in PBS in one of the tubes labeled with the patient identifiers. Ensure the patient sample is well mixed prior to making the cell suspension. Refer to procedure, "Preparing a 2-4% Cell Suspension for Testing."
4	Place 1 drop of the test cells in each tube. A. Place 1 drop of the patient cell suspension in the tube labeled with the patient identifiers. B. Place 1 drop of "positive control" in the tube labeled "POS." C. Place 1 drop of "negative control" in the tube labeled "NEG."

Step	Action
5	Add 1 drop of "Anti-D Reagent" to each of the 3 tubes and gently mix. Be sure to use the "Anti-D Reagent" that is supplied with the kit.
6	Incubate the tubes for 5 minutes (± 1 minute) at room temperature (18-30°C).
7	Wash the tubes a minimum of 4 times in PBS and decant the last wash completely. Refer to procedure, "Manual Wash Technique."
8	Add 1 drop of "Indicator Cells" to each tube and mix well by gently shaking the tube.
9	Serofuge for the AHG time listed on the serofuge.
10	Resuspend the cell button completely and examine microscopically for mixed-field agglutination. A. A minimum of 5 microscopic fields must be looked at. B. Use 100x magnification. **Note:** A. Initial microscopic examination can be carried out either in the tube **or** on a microscope slide. B. If clumps are seen in the tube, the contents should be transferred to a microscope slide so that the number of clumps per low-powered field can be evaluated.
11	Immediately record results of patient and controls on the appropriate worksheet. Enter the patient results in the LIS. For WAH patients, fax a copy of the results to the pharmacy at 301.891.6458.
12	If the fetal cell screen is positive A. Notify the patient care area and tell them additional vials of RhIG may be indicated. B. Document the phone call in "Blood Order Processing." C. Order a Kleihauer-Betke test per SOP. D. Note: Perform weak D testing on the mother if the fetal cell screen is strong positive and the Kleihauer-Betke is negative. a. No further investigation is needed if the mother is weak D positive. b. Notify a supervisor or group lead if the weak D test on the mother is negative.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Results and Repeat Criteria

1. Negative = Four or fewer clumps of agglutinated red blood cells observed in five low-power fields. A negative test indicates that a fetal bleed less than 30 mL of whole blood has occurred and 1 vial of RhIG will provide the appropriate prophylaxis.

   Positive = Five or more clumps of agglutinated red blood cells observed in five low-power fields (≥5 clumps / 5 LPFs). A positive test indicates that a fetal bleed greater than 30 mL of whole blood has occurred and additional RhIG is needed. Reflex a Kleihauer-Betke test to quantitate the fetal bleed volume and determine the appropriate dose of RhIG.

2. A positive and negative control is run with each batch of patient specimens tested. The positive control must be positive and the negative control must be negative for the test results to be valid. The test must be repeated if either control does not yield expected results. Notify a supervisor is problems persist.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

N/A

11. EXPECTED VALUES

11.1 Reference Ranges

None established

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Rh_o (D) Immune Globulin (RhIG) is used to prevent isoimmunization in the Rh negative individual exposed to Rh positive blood as a result of a fetomaternal hemorrhage. A full dose (300 µg) is sufficient to counteract the immunizing effects of 15mL of D-positive red cells; this corresponds to approximately 30 mL of fetal whole blood. The fetal bleed test is a screen to detect a feto-maternal hemorrhage of an amount greater than that covered by a standard dose of RhIG.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

1. The test must be performed on the blood of an Rh-negative mother who delivered an Rh-positive infant.
   a. If the infant is weak D positive, false-negative results may occur.
   b. If the mother is weak D positive, false-positive results may occur.
   c. If the mother is Rh-positive or weak D positive, false-positive results will occur.
   d. If the infant is Rh-negative, false negative results will occur.

2. In cases of ABO incompatibility between mother and child, the mother's natural ABO antibodies may destroy any fetal cells in the maternal blood specimen before testing is performed. This is true for any method of detecting fetal cells in the maternal blood.

3. Failure to carry out the washing stages of the test procedure properly may give rise to a false-positive test result due to agglutination of the indicator cells by free anti-D remaining in the test system.

4. A false-positive test result may occur if the maternal red blood cells have a positive direct antiglobulin test due to an autoantibody capable of reacting with the indicator cells.

5. A positive test result does not of itself provide evidence that an increased dose of RhIG is required to protect the mother from producing anti-D, but merely indicates that a larger-than-normal feto-maternal hemorrhage may have occurred. A quantitative procedure is required to determine the volume of feto-maternal hemorrhage.

6. The reactivity of the red blood cells may tend to diminish over the dating period.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

Contaminated reagents, improper incubation time, temperature, centrifugation, examination for agglutination, and deviation from the procedure may give rise to false test results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental, Health and Safety (EHS) Manual to the learn requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.

16. RELATED DOCUMENTS

SOP: Sample Specifications for Blood Bank Testing
SOP: Preparing a 2-4% Cell Suspension for Testing
SOP: Manual Wash Technique
SOP: Order Entry, Entering Orders in the GUI System
Current package insert, Fetal Bleed Screening Test

17. REFERENCES

1. Roback, J.D., Grossman, B.J., Harris, T., and Hillyer, C.D. 2011. Technical Manual of the AABB, 17th ed. AABB Publishing, Bethesda, Maryland.
2. Standards for Blood Banks and Transfusion Services, 2014. AABB, 29th ed. AABB Publishing, Bethesda, Maryland
3. FMH RapidScreen Manufacturer's Instructions, Insert code 3047-1, Revised 3/12. ImmucorGamma: Norcross, GA.

18. REVISION HISTORY

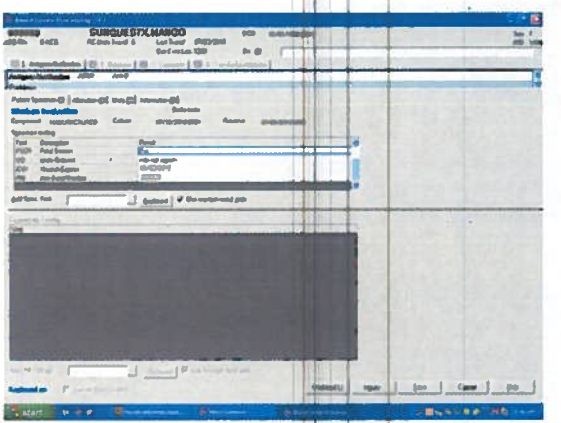
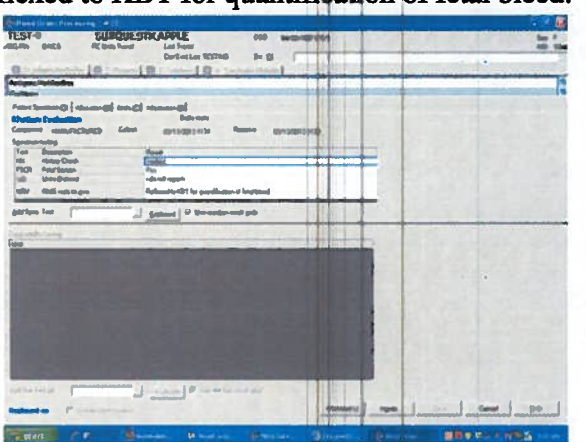
Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP WAH.BB49.002, SGAH.BB51.002		
000	3.18.2013	19	App A—Updated LIS resulting to include NRV field. App C—Added additional lines (larger bleed dosing) to RhIG dosing chart	SCodina	NCacciabeve
001	5.19.2014	8 19 Footer	History check: updated wording for clarity. Added requirement to fax WAH results to pharmacy. Deleted appendix on how to order the KBT and refer user to the SOP. Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	SCodina	NCacciabeve

19. ADDENDA

A. Entering RHOG Results in the LIS
B. Determining RhIG Dosage

Appendix A

Entering RHOG Results in the LIS

Step	Action
1	Access SunQuest function "Blood Order Processing."
2	In the "Lookup by" prompt, click on the dropdown menu and select "Patient ID."
3	In the "Value" prompt, type the patient's medical record number and click on the "Search" button.
4	If more than one patient appears, select the correct patient by clicking on the name.
5	Click on the "Search All" button.
6	Click on the "RHOG" specimen.
7	Enter the fetal cell screen result in the "FSCR" entry area. A. Enter "P" for positive. B. Enter "N" for negative. 
8	In the "NRV" field, enter the number of RhIG vials to give. A. If the fetal cell screen result was negative, enter "1" in this field. B. If the fetal cell screen results was positive, enter the mnemonic ";RKBT" which expands to "Reflexed to KBT for quantification of fetal bleed." 
8	Click on the "Save" button.

Appendix B

Determining RhIG Dosage

RhIG Dosing Based on Kleihauer-Betke Results

% Fetal Cells	Vials to Inject
<0.3	1
0.3 – 0.8	2
0.9 – 1.4	3
1.5 – 2.0	4
2.1 – 2.6	5
2.7 – 3.2	6
3.3 – 3.8	7
3.9 – 4.4	8
4.5 – 5.0	9
5.1 – 5.6	10
5.7 – 6.2	11
6.3 – 6.8	12
6.9 – 7.4	13
7.5 – 8.0	14
8.1 – 8.6	15

Calculating the Dose of RhIG Manually (Reference Only)

Step	Action
1	One 300-µg vial of RhIG is needed for a bleed of 30 mL of fetal whole blood or 15 mL of red blood cells.
2	To calculate the volume of fetal hemorrhage, use the formula: Fetal Hemorrhage mL (whole blood)= $\frac{\text{Number of Fetal Cells Counted}}{\text{Total Cells Counted}} \times 5000\ \text{mL}$ Note: 5000 mL is an estimated maternal blood volume. The actual maternal blood volume can be entered here if known.
3	Calculate the number of RhIG vials needed by dividing the volume of fetal hemorrhage by 30 mL. Always add 1 additional vial of RhIG to account for variation in the Kleihauer-Betke test results. A. If the decimal point is <0.5, round down to the nearest whole number and add 1 extra vial. B. If the decimal point is ≥0.5, round up to the nearest whole number and add 1 extra vial.