University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 03-11-16 Revision Effective Date:	Number: PC-0026.01

TITLE: Fetal Maternal Hemorrhage RapidScreen for RhIG Evaluation

PURPOSE:

To provide instructions for evaluating patients for Rh Immune globulin administration and performance of the Fetal Maternal Hemorrhage (FMH) RapidScreen if indicated. The FMH RapidScreen is used for the detection of D-positive red blood cells in D-negative mothers post-delivery or in prenatal samples following a potentially sensitizing event. Results are used to assist in the determination of Rh Immune Globulin (RHIG) dosage in eligible patients.

PRINCIPLE AND CLINICAL SIGNIFICANCE:

Principle

The following tests are includes as part of the Rh Immune Globulin Evaluation: ABO/Rh, FMH Rapid Screen and antibody screen must be added manually if not performed within 72 hours prior to releasing RhIG.

The FMH RapidScreen uses a suspension of red cells from an Rh negative mother or pregnant woman >20 weeks gestation, which is incubated with reagent serum containing Anti-D, and then washed to remove unbound antibody. Reagent R2r cells are added prior to centrifugation and then examined under the microscope for rosettes. If a sufficient volume of Rh positive fetal cells have entered the circulation, the R2r cells will form rosettes around the minor cell population of Rh positive cells and will be detected upon microscopic examination.

Clinical Significance

In the presence of a large feto-maternal hemorrhage, the standard 300ug dose of RhIG could be insufficient in preventing sensitization to the D antigen. A positive FMH RapidScreen test must be followed up with a quantitative assay to determine the appropriate dosage of Rh Immune Globulin required to prevent sensitization.

POLICIES:

- RhIG Evaluation is required on Rh negative women (not previously sensitized to the D antigen) following the delivery of an Rh positive infant or when the type of the infant is unknown
- Positive FMH RapidScreen testing is referred to Northwest Hospital Hematology for Kleihauer-Betke (quantitative) testing and determination of the appropriate RhIG dosage
- Patients identified as weak D categories I, II or III can be safely considered as Rh positive and are not candidates for RhIg - Consult with TSL MD if eligibility for RhIg is unclear
- Send out for KB testing on weak D positive (non-category I, II, III or unassigned)
- The FMH RapidScreen should not be performed if either the mother or infant is weak D positive. The mother's specimen should be referred for Kleihauer-Betke (KB) testing at Northwest Hospital if fetal bleed testing would otherwise be indicated.

SPECIMEN REQUIREMENTS:

- EDTA sample collected post-delivery (or other potentially sensitizing event) stored at 1-10°C if not tested upon receipt.
- EDTA specimen should not be stored for longer than 48 hours prior to testing, but should be tested as soon as possible to allow administration of Rh-Immune globulin within 72 hours of the sensitizing event
- Do not use grossly hemolyzed specimens
- See SOP *Specimen Acceptability*

REAGENTS/SUPPLIES/EQUIPMENT:

Reagents:	Supplies:	Equipment:
• FMH RapidScreen kit • Blood Bank Saline	• 12 x 75 mm test tubes • Pipettes • Microscope slides	• Calibrated serologic centrifuge • Calibrated cell washer • Microscope

QUALITY CONTROL:
Positive and Negative controls are tested with each batch test run and must react as expected prior to reporting results

INSTRUCTIONS:

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Determining RhIg Candidacy

STEP	ACTION
1	Perform the ABO/Rh on the maternal post-partum specimen or following a potentially sensitizing event (refer to SOP *ABO/Rh Manual Tube Method*) **If patient is** / **Then** Rh Positive → • Patient is not a candidate for Rh Immune Globulin • Go to section "Results Reporting in Sunquest" Rh Negative → Go to next step
2	Review Cord Blood log to determine infants MRN for look-up in SQ BBI **If infant is** / **Then** Rh Negative → • Patient is not a candidate for Rh Immune Globulin • Go to section "Results Reporting in Sunquest" Rh Positive or type unknown → Go to next step
3	Review the patient's current antibody screen results **Note:** Anti-D will be assumed passive (due to RhIg administration) unless careful patient history review indicates no recent RhIg administration **If the patient** / **Then**

STEP	ACTION	
	Has a history of immune anti-D (not from RhIg)	• Patient is not a candidate for Rh Immune Globulin • Go to section "Results Reporting in Sunquest"
	Has a current antibody screen on file (within 72 hours) with no anti-D	Go to next step
	Does NOT have a current antibody screen on file	Add an antibody screen to the battery by typing AS in the 'Add Spec. Test' field. Perform and result according to the SOP *Antibody Screen Testing*

STEP	If	Then
4	All the following apply: • Patient is Rh negative • Infant is Rh positive or type unknown • Patient does not have immune anti-D • Patient is >20 weeks gestation	Go to section "Performing FMH RapidScreen"
	Patient is <20 weeks gestation	• Patient is a candidate for a single 300 ug dose. The fetal blood volume does not warrant more than one dose of Rh Immune Globulin • Go to section "Results Reporting in Sunquest"

Performing FMH RapidScreen

STEP	ACTION
1	Prepare an approximate 3-4% red cell suspension of the well-mixed maternal blood in saline
2	Label 12 x 75 test tubes for the following (refer to SOP *Labeling Tubes*) • Patient specimen • Positive control • Negative control
3	Place 1 drop of the anti-D reagent from the FMH RapidScreen kit in each tube
4	Add one drop of the patient's 3-4% red blood cell suspension to the appropriate tube
5	Add one drop of each well mixed control to the appropriate tubes
6	Mix well and incubate 5 (±1) minutes at room temperature (18-30°C)
7	Wash test tubes 4 times with blood bank saline
8	Add 1 drop of well-mixed Indicator Cells to each tube and mix well
9	Centrifuge for time posted for "saline"
10	Re-suspend red blood cell button completely and examine 5 low power fields (lpf) microscopically for rosettes. This can be done in the tube or by transferring the

	contents of the test tubes onto a microscope slide. **If agglutination is** / **Then** Present: • Transfer contents to a slide for evaluation • Go to next step NOT present: Go to section 'Interpretation for FMH Testing'
11	Observe and count the number of rosettes in 5 low power fields
12	Go to section 'Interpretation for FMH Testing'

CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES

Interpretation for FMH Testing

If observed in 5 lpf	Then	RhIG Eligibility
≥5 rosettes	• Test is positive • Order a KB on positive patient specimens and send to Northwest Hospital for testing	Yes, dose based on quantitative KB results
≤ 4 rosettes	Test is negative	Yes, standard 300ug dose

Note: Positive control must be positive and negative control must be negative for the test to be valid

Results Reporting

STEP	ACTION
1	Select Blood Order Processing (BOP) function in Sunquest and open order "Rh Immune Globulin Evaluation" (abbreviated "RHEV") **NOTE:** Results are recorded on the Phenotyping Worksheet during Sunquest downtime
2	Click "Patient Specimen" tab and enter history check
3	Enter the reaction results for ABO/Rh in the test grid and interpret according to SOP *ABO/Rh Manual Tube Method* **If patient is** / **Then** Rh Positive or Weak D Positive - Category I,II or III: • Credit the billing by adding FETBCR in the 'Add Spec. Test' field • In the Rh Eligibility field, add **;;Not Eligible** • No further action required Rh Negative: • Enter the baby's Medical Record Number in the appropriate field (if available) by typing **;;(MRN)** **EXAMPLE:** ;;U123456 • In the Rh Eligibility field, add **;;Eligible** • Go to next step

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If FMH testing is	Then
Not Indicated	• Credit the billing by adding FETBCR in the 'Add Spec. Test' field • No further action required
Indicated	• Go to next step

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Enter the lot number and expiration date of the FMH RapidScreen Kit
- Add ;BBCS in the 'Add Spec. Test' field
- In the BBCS field, type **;;**FMHLOT followed by the kit's lot number and expiration date

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Click on Fetal Bleed Screen test result box and press the <Home> key to report FMH RapidScreen results in the test grid

If	Enter	Sunquest Key	Interp.	Sunquest Key
> 4 clumps in 5 lpf	>4	>	Positive	P
< 5 clumps in 5 lpf	<5	<	Negative	N

FSP = Positive Control
FSN = Negative Control
FSPAT = Patient Results

NOTE: If controls don't react as expected or the patient result is strongly positive, repeat test or perform weak D testing on the patient sample. If the problem persists, enter "Inconclusive' (Sunquest Code: **D**) in the interpretation field.

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If FMH test is	Then
Negative	No further action needed
Positive, or inconclusive	Go to next step

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Send the sample to Northwest Hospital for Kleihauer-Betke testing
- In the 'Add Spec. Test' field, add ;BBC, then type ;;Referred for KB Testing
- Go to the 'Order Entry' function on Sunquest, and order KBAE (Kleihauer-Betke Acid Elution) on the sample
- Relabel the sample with the new CID for KBAE without covering with original CID and Accession #
- Send the sample to the Core Lab Reference Lab send out area (Tube station 220)
- Notify the TSL MD on-call regarding the positive FHM RapidBleed

CALIBRATION:
NA

NOTES AND LIMITATIONS:
- Weak D testing can be added by entering ;DU in the 'Add Spec. Test' field
- The FMH RapidScreen test is not valid when the infant or maternal sample is weak D positive and the Kleihauer-Betke test is recommended fetal bleed determination

- Strong positive reactions in the FMH RapidScreen may be seen in weak D positive mothers. Weak D testing should be performed to rule out maternal weak D antigen prior to reporting results if strong positive results in the FMH Rapid Bleed are obtained.
- The number of rosettes observed on a slide does not correlate with the number of positive cells present in the maternal blood sample or in the positive control
- Careful adherence to the procedure is important. Variations in centrifuge speeds and time, incubation temperature and time, efficiency of washes, etc. may result in erroneous test results
- False positive test results may be obtained in the FMH RapidScreen if the maternal cells have a positive direct antiglobulin test
- The presence of ABO incompatibility between mother and fetus may result in a false negative test result. If ABO incompatible fetal cells are cleared from the maternal circulation at an accelerated rate, the presence of a large fetal-maternal hemorrhage may be obscured
- Reactivity of the reagent red blood cells may diminish over the dating period
- The 3-4% suspension of RBCs can be prepared by using volume estimation with comparison to the reagent red cells for visual verification

REFERENCES:

- Technical Manual. Bethesda, MD: AABB Press, current edition
- FMH RapidScreen Package Insert. Norcross, GA: Immucor Gamma; current version

APPENDICES:

NA

RELATED DOCUMENTS:

SOP Specimen Acceptability
SOP Weak D Manual Tube Testing Procedure
SOP ABO/ Rh manual testing
SOP Antibody Screen

UWMC SOP Approval:			
UWMC CLIA Medical Director	Mark H. Wener, MD	Date	
Transfusion Service Manager	Deanne Stephens	Date	
Compliance Analyst	Christine Clark	Date	
Transfusion Service Medical Director	John R. Hess, MD	Date	
UWMC Biennial Review:			
		Date	
		Date	