|  |  |  |  |
| --- | --- | --- | --- |
|  | **FMH Rapid Screen****(Fetal Maternal Bleed Screen)**BB.ROUTINE.1066 | **Dept:**  | 324311 |
| **Dept Name** | Blood Bank |
| **Effective Date:** | 7/24/19 |
| **Revised Date:** |  |
| **Name & Title**: CLIA Laboratory Medical Director | **Contact:** | Julie Simmons/Christina Warren |
| **Signature:** | G. Pomper | **Date:** | **7/23/19** |

****

**1. General Procedure Statement:**

1. **Purpose:** FMH RapidScreen is intended for use in the detection of D-positive red blood cells in

D- negative mothers.

Rh immunization in pregnancy most commonly results from the fact that, at delivery, a variable volume of fetal blood enters the maternal circulation when the placenta separates from the uterine wall. This may result in the production of maternal alloantibodies directed against fetal red cell antigens. The presence of D-positive fetal cells in maternal circulation may result in maternal antibody production in D negative mothers. In most cases, D negative mothers delivering D positive infants can be protected from producing anti-D by the administration of Rh-Immune globulin within 72 hours of delivery. Massive feto-maternal hemorrhage may be one cause for the failure of prophylaxis. The standard 300ug/dl dose of Rh-Immune globulin is sufficient to suppress Rh immunization providing no more than 15 mL of fetal red blood cells (equivalent to 30mL of whole blood) have entered the maternal circulation. AABB Standards require testing postpartum samples from D-negative females to detect the presence of a large fetal bleed (greater than 30ml) that may necessitate additional Rh-Immune globulin prophylaxis.

 **B.** **Responsible Department/Scope:**

1. Procedure owner/Implementer: Julie H. Simmons/Christina S. Warren
2. Procedure prepared by: Julie H Simmons
3. Who performs procedure: Department staff/management

 **C. Definitions:**

**FMH RapidScreen:** Immucor test kit used for the detection of D-positive red cells in D-negative mothers

 **RHIG:**  Rh Immune globulin

**Fetal Hgb test:** a test used to determine the presence of fetal hemoglobin in a maternal sample. There are two methodologies: KB (acid elution) and flow cytometry

**Fetomaternal Bleed, flow cytometry:** Beaker test for fetal hemoglobin testing and calculation of RHIG for mother. Resulted as fetal bleed in mLs and # vials of RHIG recommended. Test routinely used by WFBMC.

**KB:** Kleihauer Bekte acid elution stain, manual method to distinguish fetal hemoglobin in the maternal sample. Not routinely performed by WFBMC.

 **SCC:** Blood Bank computer system

 **FBSRT:** Fetal Bleed Screen (Rosette test) – SCC test to be used to result the FMH Rapid Screen

 **FBS:** SCC test- Fetal Screen test used at Wilkes, High Point and Lexington ONLY.

 **MRN:** Medical Record Number

 **INVAL:** SCC result for Invalid results that need to be confirmed by flow cytometry

**Indicator cells:** 0.5% suspension of O positive red cells used to determine if anti-D is present

**Positive control:** 2-4% suspension of approximately 99.4% O Neg + 0.6% O Pos red cells

**Negative control:** 2.4% suspension of O Neg red cells

 **D. Sections**:

1. Performing FBSRT Test
2. Resulting FBSRT Test in SCC
3. Lot to Lot testing

 **E. Protocol:**

1. Blood Bank at Wake will result the fetal bleed test in SCC called, “FBSRT” and not the “FBS” that is available for other sites. This is part of the RHIG Candidacy Test ordered by the provider.

*Refer to: RhIG Candidacy Test: BB.Routine.1067*

1. The **FMH RapidScreen** should be performed when:
2. Rh negative patient whose pregnancy has terminated up to 13 weeks gestation until term and fetus/infant status is Rh positive (D positive or weak D positive) or unknown.
3. Patient is not actively immunized to the D antigen. If anti-D is detected in the patient’s plasma:
4. The patient is NOT considered to be immunized when the anti-D activity is caused by passive antibody due to previous administration of Rh Immune globulin
5. The patient is considered immunized when anti-D in the plasma is not due to previous Rh Immune globulin administration.
6. RH Immune globulin is still necessary in the presence of other alloantibodies besides anti-D.
	* i.e. Patient is Rh negative with only anti-K – should get Rh Immune globulin
7. Rh Immune globulin must be administered within 72 hours of delivery or possible exposure or immunizing event. If for some reason RhIG was not given within this 72 hour period, later administration should NOT be withheld. Forward this information to the medical Director for review if this occurs.
8. The test *must* be performed on the blood of a known D-negative mother of a recently delivered D-positive child.
	1. The baby’s name and MRN will be on the RHIG Candidacy test requisition.
9. If there is no baby information on the requisition but the statement: “No information on baby” this means that the baby was either still born or adopted. Give to Management or the Medical director to investigate.
	1. When there are multiple babies delivered, if at least one of them is D-Positive the FBSRT must be performed on the mother’s sample.
10. A negative FMH Rapid Screen indicates that a standard dose of 300ug of RHIG is sufficient to prevent immunization to the D antigen in 99% of cases.
11. **False positive** results may occur if the washing stage of the test procedure is not done properly.
12. **Reagents:**
	1. New lots of kits (reagents) require lot to lot testing before use.
	2. Reagents may be interchanged between lots as long as they are in date.
13. **Fetomaternal Bleed, flow cytometry test** must be performed on mother to detect fetal hemoglobin if:
	1. If the infant’s red blood cells possess a weak D or partial D antigen.
14. The FBSRT test may not detect a feto-maternal hemorrhage exceeding 30 mL of whole blood when the weak D test on the infant is positive.
	1. If the infant’s DAT is positive.
	2. If the mother is D-positive, including weak D, strong agglutination provides no information about the extent of feto-maternal hemorrhage.
	3. If the FMH Rapid Screen is positive, this indicates that a large feto maternal hemorrhage has occurred and a Fetal Hgb test should be performed to accurately calculate the dose of RHIG.
	4. If the DAT is positive on the maternal sample, FBSRT false positive results may occur in the presence of a positive DAT due to an autoantibody capable of reacting with the indicator cells.
15. **The Fetomaternal Bleed, flow cytometry test** (Fetal hgb detection) is performed at WFBMC in Core lab and Mayo (as a send out test).
	1. When the flow cytometry test at WFBMC is not available for some reasons, send specimen out to Mayo Clinic for flow cytometry for Fetal hgb test via Send Outs in core lab.
	2. Blood Bank will divide their sample and send 1mL of well mixed sample to core lab for testing (or sending out to Mayo if necessary). Label with the Beaker label that prints in BB when ordered by Epic.
	3. The Fetal Hgb flow test will be automatically ordered by Epic when the FBSRT is resulted as POS or INVAL in SCC.
	4. All samples being sent for flow testing (whether in core lab or Mayo) must be logged on the log sheet: *FBSRT samples for Fetal Hgb Log*
	5. The Fetomaternal Bleed, flow cytometry test results will print in Blood Bank upon completion. (this is only for the test performed at WFBMC. The results from Mayo will not automatically print)
	6. The results will print on the front desk Epic add-on printer

**2. Procedure:** **I.** **Performing FBSRT- Fetal Bleed Screen (Rosette Test) on Mother’s sample**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 or 12x75 tubes, plastic pipets, saline, microscope slides, PPE

Reagents: FMH RapidScreen Test Kit - Immucor

Equipment: Centrifuge, 37C Incubator, Microscope with low power (100-150x)

Specimen Requirements: EDTA (purple or pink top) – Collected from the mother post-delivery or hemorrhage.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Prepare a 2-4% suspension of the well-mixed maternal red blood cells to be tested in isotonic saline.*** 1. Label a 10 x 75 or 12 x75 test tube with patient’s name and MRN or a label with this information.
	2. Mix tube containing maternal red blood cells (collection tube).

**NOTE: It is extremely important to mix the specimen tube to get an accurate result.*** 1. Add 1 to 2 drops of maternal red blood cells to the labeled 10 x 75 or 12x75 tube.
	2. Add isotonic saline to create a 2-4% red cell suspension.
	3. Visually compare color of suspension with that of a 2-4% commercial reagent red cell suspension.
	4. If it appears <2%, add patient red cells to achieve a 2-4% suspension.
	5. If it appears >4%, add saline to suspension to achieve a 2-4% suspension.
 |  |
| **2.0** | **Perform ABO/Rh forward and reverse testing on maternal specimen.***Refer to Routine: ABORh Testing*2.1 Enter results into SCC.2.2 Perform weak D typing if patient is Rh negative.2.3 Proceed to perform the FMH Rapid Screen if patient is Rh negative.2.4 If the patient is 2-4+ Rh positive check the prenatal Rh result. Consult management or medical director for further direction. |  |
| **3.0** | **Label three (3) 10x75 or 12x75 test tubes:**1. Maternal (label with small label or a minimum of first three letters of patient’s last name.)
2. Positive control
3. Negative control
 |  |
| **4.0** | **Gently mix the positive and negative control cell reagent vials.** |  |
| **5.0** | **Add the following reagents/cells to each tube as indicated below.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cells/Reagents** | **‘Maternal’ Tube** | **‘Positive’****Control Tube** | **‘Negative’****Control Tube** |
| Add 1 drop of  | ‘maternal’ 2-4% rbc suspension | ‘Positive’Control Cell | ‘Negative’Control Cell |
| Add 1 drop of  | Anti-D reagent | Anti-D reagent | Anti-D reagent |

Note: The anti-D reagent used in this test is the anti-D in the FBS kit. Anti-D on reagent rack cannot be used for this test.  |  |
| **6.0** | **Mix each tube well.** |  |
| **7.0** | **Incubate tubes for five (5) ± 1 minute(s) at Room Temperature (18-30C).** |  |
| **8.0** | **Wash tubes four (4) times with 0.9% saline in cell washer.**8.1 Thoroughly decant the saline after each wash if washing manually. |  |
| **9.0** | **Add 1 drop of indicator cells to each tube (maternal tube, positive control and negative control).**9.1 Gently mix by shaking the tube.  |  |
| **10.0** | **Centrifuge immediately for 15 seconds at 3400-3600 rpm or a time appropriate to the calibration of the centrifuge.** |  |
| **11.0** | **Resuspend the red blood cell button and examine five (5) low power fields microscopically for mixed field agglutination using approximately 10x magnification.** 11.1 If agglutinates are seen in the tube, then the contents should be transferred to a microscope slide so that the number of agglutinates per low-power field can be evaluated. |  |
| **12.0** | **Interpret test and control results.**

|  |  |
| --- | --- |
| **Interpretation** | **Criteria** |
| **Positive Test** | A total of five or more agglutinates are observed in five low-power fields. |
| **Negative Test** | A total of four or fewer agglutinates are observed in five low-power fields.  |
| **Invalid Test** | Positive control has four or less agglutinates per five low –power fields and/or Negative control has five or more agglutinates per five low power fields. |

12.1 Positive Control should be positive12.2 Negative Control should be negative |  |
| **13.0** | **Determine if additional testing is required:**

|  |  |
| --- | --- |
| **Test Result** | **Additional steps needed** |
| **Positive FBSRT** | Perform DAT on FBSRT (maternal) sample.DAT must be negative to report test as Positive.If DAT is positive, report FBSRT as “INVAL”. The Fetal Hgb by flow cytometry testing is automatically ordered on mother. |
| **Negative FBSRT** | Check to see if infant is Weak D positive.If Weak D positive, report FBSRT as “INVAL”. The Fetal Hgb by flow cytometry testing is automatically ordered on the maternal sample. |
|  |  |

 |  |
| **14.0** | **Enter results into SCC.**14.1 Refer to Section II: Resulting FBSRT Test in SCC. |  |
| **15.0** | **Determine next step based on patient’s results:**

|  |  |
| --- | --- |
| **Interpretation** | **Next Step(s)** |
| **Negative** | 1. Patient meets criteria for administration of RHIG, one (1) vial of RHIG may be issued by pharmacy.
2. No further testing is indicated.
 |
| **Positive or****INVAL** | 1. Report all positive test to management and Medical Director.
2. A ‘Fetomaternal Bleed, Flow Cytometry’ test will automatically be ordered in Epic when the FBSRT is resulted as POS or INVAL
	1. This is to determine the volume of fetomaternal hemorrhage and the dose of RhIG to be administered to the mother.
3. Notify appropriate department:
	1. **For in house** flow testing notify Hematology (# 3-8162) that a fetomaternal flow test has been ordered and that you will be tubing a sample to the core lab.
	2. **For send out** flow testing notify Sendouts (# 6-4965) that a fetomaternal flow test has been ordered and that you will be tubing a sample to the core lab
4. Pour off an aliquot of 1mL into an aliquot tube with a twist top (for security against spillage).
	1. Label tube with the Beaker label that printed for the flow test and place in sample bag. (label prints to label printer next to SCC label printers: MCBLDBNKZBRA168)
	2. For the **send out test only**: receive the test in Beaker

Refer to *Specimen Receipt in SCC and Epic: BB.FD.1006** 1. Tube aliquot specimen to Core lab.
1. Fill out log sheet on the bridge “FBSRT samples for Fetal Hgb Log.” The small taglet from the Beaker label can be used.

Refer to *Attachment A: FBSRT samples for Fetal Hgb Log*1. Results of the fetomaternal bleed, flow test:
	1. When performed **in house** they will print in Blood Bank at the front desk printer when complete.
	2. When performed as a **sendout** the results will need to be manually printed from Epic.
2. See *Attachment B: Printing Lab Results in Epic/Beaker*
3. Place the printed fetomaternal bleed, flow test results on the Medical Director’s desk for review and complete the *FBSRT samples for Fetal Hgb log (Attachment A)*
4. For questions regarding RHIG dosage see

*Attachment B:* *Calculating Dosage – Rh Immune Globulin*.*Resurrected* |

 |  |

**2. Procedure:** **II.** **Resulting FBSRT- Fetal Bleed Screen (Rosette Test) on Mother in SCC**

 Chemical Risk Assessment: Low

 Biological Risk Assessment: Low

 Protective Equipment: Lab coat, gloves

 Supplies: NA

 Reagents: NA

 Equipment: Computer with SCC

 Specimen Requirements: NA

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Go to SCC TEST>Patient>Orders>Modify to order test.**1.1 Enter Patient’s MRN.1.2 Select test from current visit.1.3 F12 to accept.1.4 F12 to review history if prompted.1.5 Select FBSRT from ‘Tests’ dropdown menu.1.6 F12 to accept and Yes to accept changes. NOTE: The FBSRT test will be a part of the RHIG candidacy work up and will be ordered by the floor at Go Live.  |  |
| **2.0** | **Go to Patient>Orders>Results.**2.1 Enter Patient’s MRN and F12 to accept.2.2 Double click of FBS (Rosette) test.2.3 Select rack number that is being used from drop down if it does not default.2.4 Enter the patient and control results – refer to table below.1. The following are the codes in SCC for resulting:
	1. FSC – Maternal specimen result
	2. PCT – Positive Control Result
	3. NCT – Negative Control Result
	4. DATIn – DAT interp on sample
	5. BWkD – Baby’s Weak D interp

|  |
| --- |
| **LOGIC TABLE FOR RESULTING IN SCC** |
| **FSC** | **PCT** | **NCT** | **DATIn** | **BWkD** | **INTRP** | **Comments** |
| + | + | - | + | NT | **INVAL** | Fetal Hgb test Required. DAT can cause a false positive.  |
| + | + | - | - | NT | **POS** | Fetal Hgb test required to quantitate the bleed to determine RHIG dose. |
| - | + | - | NT | - | **NEG** | Test negative. One dose of RHIG should be sufficient.  |
| - | + | - | NT | + | **INVAL** | Fetal Hgb test required – Test may not detect Weak D positive fetal cells.  |

* 1. An interpretation of INVAL or POS requires a Fetal Hgb test be performed to verify and quantitate a fetomaternal bleed.
1. A result of INVAL or POS will prompt the reflex order (in Beaker) of the Fetomaternal bleed, flow cytometry test. Beaker labels will print in Blood Bank on the label printer: MCBLDBNKZBRA168.
2. An aliquot must be poured off into an aliquot tube (5mL cryo vial) with a minimum of 1mL well mixed whole blood. Label with the Beaker label that printed from the previous step. Ensure that the lid is on securely.
3. Place a Beaker label or write patient name/MRN on the “FBSRT samples for Fetal Hgb” log sheet on the bridge. Record the FBSRT interpretation, date and time sent to Core lab and your initials.

*Refer to Attachment A: FBSRT Samples for Fetal Hgb Log*1. Place labeled aliquot tube and extra labels in a biohazard sample bag and tube to the core lab, tube station 21.
2. Call Hematology (#38162) to inform them of the arriving sample.
3. The results of the Fetal Hgb test will print in Blood Bank on the front desk printer. Place on the medical director’s desk and record on the “FBSRT samples for Fetal Hgb” log sheet on the bridge.

*Refer to Attachment A: Refer to FBSRT Samples for Fetal Hgb Log*2.6 An interpretation of NEG requires no further steps once resulted.2.7 If the positive and/or negative controls do not work, then the test is invalid and must be repeated. |  |
| **3.0** | **Determine next step based on patient’s results:**

|  |  |
| --- | --- |
| **Interpretation** | **Next Step(s)** |
| **Negative** | Patient meets criteria for administration of RHIG, one (1) vial of RHIG may be issued by pharmacy.No further testing is indicated.  |
| **Positive or****INVAL** | Report all positive test to management and Medical Director.1. ‘Fetomaternal Bleed, Flow Cytometry’ test will automatically be ordered in Epic when the FBSRT is resulted as POS or INVAL
2. This is to determine the volume of fetomaternal hemorrhage and the dose of RhIG to be administered to the mother.

Notify appropriate department:1. **For in house** flow testing notify Hematology (# 3-8162) that a fetomaternal flow test has been ordered and that you will be tubing a sample to the core lab.
2. **For send out** flow testing notify Sendouts (# 6-4965) that a fetomaternal flow test has been ordered and that you will be tubing a sample to the core lab

Pour off an aliquot of 1mL into an aliquot tube with a twist top (for security against spillage). 1. Label tube with the Beaker label that printed for the flow test and place in sample bag. (label prints to label printer next to SCC label printers: MCBLDBNKZBRA168)
2. For the **send out test only**: receive the test in Beaker

Refer to *Specimen Receipt in SCC and Epic: BB.FD.1006*1. Tube aliquot specimen to Core lab.

Fill out log sheet on the bridge “FBSRT samples for Fetal Hgb Log.” The small taglet from the Beaker label can be used.Refer to *Attachment A: FBSRT samples for Fetal Hgb Log*Results of the fetomaternal bleed, flow test:1. When performed **in house** they will print in Blood Bank at the front desk printer when complete.
2. When performed as a **sendout** the results will need to be manually printed from Epic.

See *Attachment B: Printing Lab Results in Epic/Beaker*Place the printed fetomaternal bleed, flow test results on the Medical Director’s desk for review and complete the *FBSRT samples for Fetal Hgb log (Attachment A)*1. For questions regarding RHIG dosage see

*Attachment B:* *Calculating Dosage – Rh Immune Globulin*. |

 |  |

1. **Procedure:** **III.** **Fetal Bleed Screen (Rosette Test) Kit Lot to Lot testing**

 Chemical Risk Assessment: Low

 Biological Risk Assessment: Low

 Protective Equipment: Lab coat, gloves

 Supplies: NA

 Reagents: NA

 Equipment: Computer with SCC

 Specimen Requirements: NA

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | Receive in the reagents upon arrival.1. Refer to: *Reagent/Supply/Form/Label Receiving Log (BB.FORMS.1083)*
2. Refer to procedure: *Receiving Reagents, Supplies, Forms, and Labels into the Blood Bank*
 |  |
| **2.0** | Test each NEW LOT number of kit that arrives with lot to lot testing.1. Test the NEW lot# Positive and Negative controls with the NEW reagent
2. Test the Current lot # Positive and Negative controls with the NEW reagent.
 |  |
| **3.0** | **Label four (4) 12x75 test tubes.**1. New Positive control: New +
2. New Negative control: New Neg
3. Curr Positive control: Curr +
4. Curr Negative control: Curr Neg
 |  |
| **4.0** | **Gently mix all of the positive and negative control cell reagent vials.**  |  |
| **5.0** | **Add the following reagents/cells to each tube as indicated below.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cells/ Reagents** | **New lot# ‘Positive’****Control Tube** | **New lot # ‘Negative’****Control Tube** | **Curr lot# ‘Positive’****Control Tube** | **Curr lot # ‘Negative’****Control Tube** |
| Add 1 drop of | New lot#‘Positive’ Control Cell | New Lot# ‘Negative’ Control Cell | Curr lot# ‘Positive’ Control Cell | Curr lot# ‘Negative’ Control Cell |
| Add 1 drop of | New lot#Anti-D reagent | New lot#Anti-D reagent | New lot#Anti-D reagent | New lot#Anti-D reagent |

**NOTE:** Curr refers to the Current lot # in use. |  |
| **6.0** | **Mix each tube well.** |  |
| **7.0** | **Incubate tubes for five (5) ± 1 minute(s) at Room Temperature (18-30C).** |  |
| **8.0** | **Wash tubes four (4) times with 0.9% saline.**8.1 Thoroughly decant the saline after each wash if washing manually. |  |
| **9.0** | **Add 1 drop of NEW LOT indicator cells to each of the four tubes.**9.1 Gently mix by shaking the tube.  |  |
| **10.0** | **Centrifuge immediately for 15 seconds at 3400-3600 rpm or a time appropriate to the calibration of the centrifuge.** |  |
| **11.0** | **Resuspend the red blood cell button and examine five (5) low power fields microscopically for mixed field agglutination using approximately 10x magnification.** 11.1 If agglutinates are seen in the tube, then the contents should be transferred to a microscope slide so that the number of agglutinates per low-power field can be evaluated. |  |
| **12.0** | **Interpret test and control results.**

|  |  |
| --- | --- |
| **Interpretation** | **Criteria** |
| **Positive Test** | A total of five or more agglutinates are observed in five low-power fields. |
| **Negative Test** | A total of four or fewer agglutinates are observed in five low-power fields.  |
| **Invalid Test** | Positive control has four or less agglutinates per five low –power fields and/or Negative control has five or more agglutinates per five low power fields. |

12.1 Positive Controls should be positive12.2 Negative Controls should be negative |  |
| **13.0** | **Record results on the worksheet.**Refer to *Attachment C:* *Receipt Testing of* *FBSRT- Lot to Lot Testing form* |  |

**3. Review/Revised/implemented:**

All procedures must be reviewed as indicated in the document change control protocol.

All new procedures and procedures that have major revisions must be signed by the CLIA Director.

All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director or designee.

**4. Related Procedures:**

Training: FMH Rapid Screen

**5. References**:

FMH RapidScreen direction circular

AABB Technical Manual, revised periodically.

**6. Attachments**:

Attachment A: Calculating Dosage – Rh ImmuneGlobulin

Attachment B: FBSRT Samples for Fetal Hgb Log

Attachment C: Receipt Testing of FBSRT- Lot to Lot Testing form

1. **Revised/Reviewed Dates and Signatures**

See Archived Document Change Control

**Attachment A: Calculating Dosage – Rh Immune Globulin**

