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# Fetal Cell Screening Using the FMH Rapid Screen Kit - Blood Bank



Document Type: Procedure

## I. PURPOSE AND OBJECTIVE:

This document will provide policies and instructions that will enable the Blood Bank staff to perform the fetal cell screen (FCS) test using the RBC Fetal Assay Rapid Screen kit that is provided by Immucor®.

## II. CLINICAL SIGNIFICANCE:

A. Rh immunization in pregnancy may result if the mother produces antibodies that are directed at antigens present on the fetal red blood cells (RBCs) but absent on the maternal RBCs. Rh negative mothers delivering Rh positive infants are most susceptible. In most cases, Rh immunization can be prevented by the administration of Rh Immune Globulin (RhIG) within 72 hours from the time of delivery. One standard 300 µg dose of RhIG is generally sufficient to prevent immunization when up to 30 ml of fetal whole blood (approximately 15 ml of RBCs) have entered the maternal circulation.

B. The Immucor RBC Fetal Assay Rapid Screen kit is a screening test used to detect the presence of Rh-positive fetal cells in Rh-negative mothers. In most cases the feto-maternal hemorrhage 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 Rh Immune Globulin (RhIG) to the mother.

C. The FCS test is a qualitative test designed to detect a feto-maternal hemorrhage of an amount greater than that covered by the standard 300 µg dose of RhIG.

# III. DEFINITIONS/ACRONYMS:

A. **BBIS:** Blood Bank Information System

B. **RhIG**: Rh Immune Globulin

C. **Rhogam**: Trade name for Rh Immune Globulin produced by Kedrion Biopharma Inc.

D. **HyperRho®**: Trade name for Rh Immune Globulin produced by Grifols.

E. **Rhophylac®**: Trade name for Rh Immune Globulin by CSL Bering.

F. **FCS**: For the purposes of this document refers to the qualitative fetal screen that is performed in the Blood Bank.

G. **FMHA:** The blood bank computer test code assigned to the final FMH report; completed after consideration of the FMH testing used for the final determination of total number of RhIG vials indicated for the patient.

H. **FMH**: Refers to the RBC fetal assay; the quantitative feto-maternal hemorrhage test that is performed in the flow cytometry laboratory at BH-Royal Oak. The orderable test code in EPIC is FRBCG (LAB292)

I. **ACDEL**: Refers to the quantitative acid elution/Kleihauer-Betke stain that is performed by the hematology departments at BH-Farmington Hills, Grosse Pointe, Troy, and Royal Oak and in Dearborn Blood Bank for Dearborn, Taylor, Trenton and Wayne. The orderable test code in EPIC is ACDEL (LAB474).

J. **Delivery**: For the purposes of this document the term “delivery” applies to all pregnancies greater than 18 weeks gestation including full-term births and pregnancies that are terminated or interrupted for any reason.



# IV. SCOPE:

## A. Indications for Fetal Cell Screening

1. The Blood Bank will assess RhIG candidacy if the mother's name appears on the Obstetrical Delivery List or if a physician orders a Rh Immune Globulin (RhIG) Evaluation.
2. The Fetal Screen may be performed on a sample from a Rh negative mother after the delivery of a Rh positive neonate.
   Note the following:
   a. The term “delivery” applies to all pregnancies greater than 18 weeks gestation including full-term births and pregnancies that are terminated or interrupted for any reason.
   b. The mother must be Rh negative and the neonate must be Rh positive in order to perform the Fetal Screen. If the Rh of the mother or neonate is weak D/Partial D, unknown, or undetermined then the Fetal Screen test may not be performed; the RBC Fetal Assay test should be performed in these cases.
   c. It is not necessary to perform the Fetal Screen test if the RBC Fetal Assay test has already been performed after delivery; e.g., if the RBC Fetal Assay test was

performed after delivery because it was ordered by the physician. The RBC Fetal Assay results may be used to assess RhIG candidacy in this case.

# B. Indications for Fetal RBC Assay

1. The Fetal RBC Assay flow cytometry test should be performed in the following cases:
    a. If the Fetal Screen performed by the Blood Bank is positive and pregnancy is greater than 18 weeks gestation.
    b. If either the maternal or neonatal are known to be a weak D/partial D.
    c. If the Rh type of either the fetal or maternal RBCs is undetermined for any reason.
    d. If there is evidence of trauma in pregnancy and delivery has not occurred the Fetal RBC Assay will be performed at physician request or if the pregnancy is greater than 18 weeks gestation.

# C. Indications for Acid Elution (Kleihauer Betke)

1. The Acid Elution (ACDEL) test is an acceptable alternative for the Fetal RBC Assay.
    a. The Fetal RBC Assay (FRBCG) is usually available Monday through Saturday (excluding holidays).
    b. The acid elution (ACDEL) stain should be ordered whenever there is a possibility that flow cytometry results will not be available within 48 hours of order. i.e. weekends and holidays.
    c. Medical Director approval must be obtained in situations where the acid elution (ACDEL) stain is specifically requested by ordering physician when the results for Fetal RBC Assay will not meet STAT time requirements.
    d. Blood Bank staff will communicate with Flow Cytometry and Hematology, if appropriate, to determine which test will be performed based on staffing, weekends, holidays, etc.



# V. POLICIES:

A. The specimen used to perform the Fetal Screen test must be collected after cessation of pregnancy (miscarriage, birth, etc).

B. Batch testing must be limited to 6 tests per batch. If workload becomes excessive, supervisory staff must be notified immediately.

C. The sample must be collected in a timely manner after delivery so that testing may be performed and RhIG may be administered within 72 hours from the time of delivery.

D. If the Fetal Screen test is positive, then the Blood Bank is responsible for sending a post-delivery maternal sample for a quantitative test. Generally, the Fetal RBC Assay test is used for this purpose, alternatively the Acid Elution (Kleihauer Betke) test can be used. For additional information, refer to Transfusion Medicine policy, *Rh Immune Globulin Evaluation - Blood Bank*.

E. Based on the results of the Fetal Screen test or the quantitative test (Fetal RBC Assay(FRBCG) or Acid Elution (ACDEL)), the Blood Bank shall determine the appropriate

number of RhIG vials to protect against Rh immunization, as described in Transfusion Medicine policy, *Rh Immune Globulin Evaluation - Blood Bank*.

F. ACDEL and FRBCG tests may be ordered by the patient's physician, or by the Blood Bank. The hematology department (for applicable sites) and flow cytometry laboratories will notify the Blood Bank whenever a positive ACDEL or FRBCG result is observed. A Blood Bank Medical Technologist will use this information to determine the appropriate dose of RhIG that should be administered, if applicable, and result the FMHA test in the BBIS with this final determination.

# VI. SPECIMEN COLLECTION AND HANDLING:

A. The test procedure requires a blood specimen collected from the mother after delivery of all products of conception. It is best to wait at least an hour after delivery to allow any fetal RBCs to mix thoroughly in the maternal circulation, but the sample should be collected as soon as possible thereafter.

B. The preferred sample is a 6ml EDTA sample with affixed identifying label. Refer to Transfusion Medicine policy, Triaging And Identifying Acceptable Samples For Testing.

C. Do not use grossly hemolyzed specimens for testing.

D. If a delay in testing occurs, the sample must be stored at 1 – 10 ° C.

E. For additional information, refer to package insert for the RBC Fetal Assay Rapid Screen kit, provided by Immucor ®.



# VII. REAGENTS:

A. Immucor® RBC Fetal Assay Rapid Kit

1. Anti-D Serum: Contains monoclonal IgM anti-D antibodies from the human/murine heterohybridoma cell line GAMA401 grown in field culture and suitably diluted in bovine albumin to achieve the appropriate level of potency.
2. Indicator cells: An approximate 0.5% suspension of group O RBCs obtained from a donor of the DcEe ($R_2r$) phenotype.
3. Positive control: A 2 - 4 % suspension of RBCs comprising approximately 99.4% group O, Rh negative cells and approximately 0.6% group O, Rh positive cells from a donor having heterozygous expression of the D antigen.
4. Negative control: A 2 - 4 % suspension of group O, Rh negative RBCs.

B. Reagent Storage and Handling

1. Do not freeze, do not dilute, do not use beyond the expiration date.
2. The FCS kit should be stored at 1 – 10 ° C when not in use.
3. The indicator cells must be well-mixed before use. If marked hemolysis and/or darkening of the cells is present, the cells should not be used.
4. The anti-D reagent should not be used if markedly turbid.
5. All blood products should be treated as potentially infectious.

6. The anti-D reagent contains 0.1% sodium azide as a preservative, which is classified as harmful.

# VIII. EQUIPMENT:

A. Microscope

B. Timer

C. Centrifuge

# IX. SUPPLIES:

A. Test tubes, 12 x 75 mm preferred

B. Disposable pipettes

C. Microscope slides

D. Isotonic saline, pH 6.5 – 7.5. Note that washing manually or using the automated cell washer is acceptable.

# X. QUALITY CONTROL (QC):



A. The positive and negative controls should be tested in parallel with each batch of patient samples.

B. Appropriate reactivity of the positive control confirms the reactivity of the indicator cells and provides an indication that the test was performed correctly. The positive control must demonstrate a positive reaction (5 or more agglutinates per 5 low-powered fields), refer to the *Interpretation* section.

C. The negative control confirms that the washing procedure removed all unbound anti-D reagent. The presence of agglutinates with the negative control suggests that the indicator cells are being agglutinated by unbound anti-D reagent (inadequate wash procedure). The negative control must demonstrate a negative reaction (4 or fewer agglutinates per 5 low-powered fields), refer to the *Interpretation* section.

# XI. PROCEDURE:

## A. Performance of the Fetal Screen

1. Label four test tubes to identify the following:
    a. Positive control
    b. Negative control
    c. Maternal cell suspension (label with patient's name)
    d. Maternal Fetal Cell Screen test; label with patient's name and "FCS," for example.
2. In the applicable test tube that was labeled in the previous step, prepare a 2 - 4% suspension of the well-mixed maternal whole blood in isotonic saline.

3. Add 1 drop of anti-D reagent to the test tubes corresponding to the patient's FCS test, to the positive control, and to the negative control.
4. Transfer 1 drop of the 2 - 4% maternal cell suspension to the tube labeled for the patient's FCS test.
5. Add 1 drop of the positive control to the correspondingly labeled tube.
6. Add 1 drop of the negative control to the correspondingly labeled tube.
7. Mix well and incubate for 5 (± 1) minutes at room temperature (18°C - 30°C).
8. Wash the RBCs 4 times ( if using 12 x 75mm test tubes) or 6 times (if using 10 x 75mm test tubes).
    a. The RBCs may be washed using the automated cell washer or washed manually.
    b. Decant completely between washes and after the last wash.
    c. Resuspend the RBCs thoroughly when adding saline for the next wash.
9. Add 1 drop of indicator cells to the dry cell button obtained after washing to the patient's FCS test, positive control, and negative control. Mix each tube well by gently shaking. **The indicator cells should be resuspended before use**. Centrifuge immediately for the calibrated immediate-spin settings of the centrifuge.
10. Resuspend the RBC button and transfer a drop to a microscope slide.
11. Examine 5 low-power fields (approximately 100x magnification) microscopically for mixed-field agglutination. Determine the number of mixed-field agglutinates observed in the 5 fields. **Results should be interpreted immediately upon completion of the test. Refer to XII. *Interpretation*.**
12. Record results in the BBIS using Transfusion Medicine Policy - SafeTrace (Blood Bank) Application.



# B. Requesting Flow Cytometry Testing

A. If the result of the FCS is positive:
    1. Order the Fetal RBC Assay (LAB292) in EPIC and collect the order in EPIC using the collection details from the specimen to be used for testing.
    Note: If it is determined that there is a possibility that flow cytometry results will not be available within 48 hours of order. i.e. weekends and holidays, then an order for ACDEL should occur. Refer to *Procedure IV.C* for further instruction.
    2. The FMHA test ID in the Rhogam Evaluation test battery in the BBIS should be left pending until the flow cytometry testing is complete. Once testing is completed the FMHA test ID is resulted with the final determination of total number of RhIG vials indicated for the patient.
    3. Place specimen on Packing List to Royal Oak Flow Cytometry.

# C. Requesting Acid Elution (Kleihauer-Betke) Testing

A. If it is determined that there is a possibility that flow cytometry results will not be available

within 48 hours of order. i.e. weekends and holidays, then:

1. Order the Acid Elution (LAB474) test in EPIC and collect the order in EPIC using the collection details from the specimen to be used for testing.
2. The FMHA test ID in the Rhogam Evaluation test battery in the BBIS should be left pending until the acid elution testing is complete. Once testing is completed the FMHA test ID is resulted with the final determination of total number of RhIG vials indicated for the patient.
3. Grosse Pointe, Farmington Hills, Troy, and Royal Oak: Transport specimen to Hematology department for testing.
4. Taylor, Trenton and Wayne: Place specimen on Packing list for testing at Dearborn Blood Bank.
5. Dearborn: Perform acid elution (ACDEL) testing in accordance with Transfusion Medicine policy, Acid Elution by Kleihaeur Betke Method - Dearborn Blood Bank.

# XII. INTERPRETATION:

A. Positive Test: After examining 5 low-power fields, if 5 or more agglutinates of RBCs are observed the test should be interpreted as positive. This indicates the presence of Rh positive fetal RBCs in possibly significant numbers in the maternal circulation. A quantitative test (Fetal RBC Assay or ACDEL) is indicated to determine whether additional vials of RhIG are needed to prevent immunization. Proceed to *Procedure XI.B* or *C*.

B. Negative Test: After examining 5 low-power fields, if 4 or fewer clumps of agglutinated RBCs are observed the test should be interpreted as negative. This indicates that a large feto-maternal hemorrhage did not occur.



# XIII. LIMITATIONS:

A. For correct interpretation of the FCS test, the test must be performed only on a sample from a Rh negative mother after the delivery of a Rh positive newborn.

1. If the neonatal RBCs are weak D or partial D positive, the test may not detect a feto-maternal hemorrhage exceeding 30 ml of whole blood. A Fetal RBC Assay or acid elution (ACDEL) must be performed in this case.
2. In some cases, maternal RBCs that are weak D or partial D positive are inadvertently tested because Corewell Health does not routinely perform weak D testing of maternal samples. A strongly positive FCS test may result if the mother is weak D or partial D positive. If this is suspected (as with all positive FCS tests) then a Fetal RBC Assay test is indicated. The maternal Rh should not be interpreted as weak D or partial D positive on the basis of a strongly positive FCS test alone. For additional information refer to Transfusion Medicine policies, Resolution of ABO and Rh Discrepancies - Blood Bank and Weak D Testing.

B. A strongly positive FCS test provides no information about the extent of feto-maternal hemorrhage.

C. If the neonate is Rh negative, a negative FCS result is expected regardless of the volume of

feto-maternal hemorrhage. A FCS should not be performed in these instances.

D. In cases of ABO incompatibility between mother and child, the maternal ABO antibodies may destroy any fetal RBCs in the maternal circulation before testing. This is true for any method of detecting fetal RBCs in maternal circulation.

E. **False-positive results may occur if the washing procedure is inadequate, or if the maternal RBCs have a positive direct antiglobulin test (DAT) due to an autoantibody capable of reacting with the indicator cells.**

# XIV. REFERENCES:


1. Package insert for the RBC Fetal Assay Rapid Screen, Immucor ® Insert code 3047-3, revision Date 03/2017.
2. College of American Pathologists, *Transfusion Medicine Checklist*, current edition.
3. AABB, *Technical Manual*, current edition.
4. AABB, *Standards for Blood Banks and Transfusion Services*, current edition.




## Approval Signatures

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