Dignity Health
Central Coast Service Area Procedure

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| **Central Coast Service Area North:** |
| [x]  Santa Maria Campus,Marian Regional Medical Center | [ ] Arroyo Grande Campus,Marian Regional Medical Center | [x] French Hospital Medical Center |
| **Central Coast Service Area South:** |
| [ ] St. John’s Pleasant Valley Hospital | [ ] St. John’s Regional Medical Center |  |

**SUBJECT**: Fetal Maternal Hemorrhage Screening Test-Immucor, Inc.

**Lab Policy Number:** 7540.BB.CC.174

# Purpose: The Fetomaternal Hemorrhage test provides a qualitative indication of a significant fetomaternal bleed in Rh(D) negative mothers, which would warrant a quantitative test to determine the dose of Rh Immune Globulin (RhIG) to be administered in order to prevent Rh(D) alloimmunization in the mother. A Fetomaternal Hemorrhage test is run on all postpartum Rh Immune Globulin (RhIG) eligible mothers to detect a fetal bleed greater than 30 milliliters of whole blood. A fetomaternal hemorrhage test should be performed on all Rh(D) negative mothers that are > 20 weeks gestation and have experienced abdominal trauma or miscarriage in order to determine the appropriate dose of RhIG to prevent Rh(D) alloimmunization.

**Table 1:** Indications for fetomaternal hemorrhage testing.

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| --- | --- |
| **If** | **Then** |
| **Mother** | **Infant** |  |
| Rh(D) positive | Rh(D) positive or Rh(D) negative | Test not indicated |
| Rh(D) negative | Rh(D) negative | Test not indicated |
| Rh(D) negative | Rh(D) positive | Test indicated |
| Rh(D) negative | Rh(D) unknown(>20 weeks gestation) | Test indicated.Note: Refer to procedure step 16 before interpretation. |
| Rh(D) negative | Weak D positive or partial D positive | Test not valid. Perform Fetal Hgb (Kleihauer Betke) |
| Weak D positive or partial D positive | Rh(D) negative | Test not indicated |
| Weak D positive or partial D positive | Rh(D) positive | Test not valid. Perform Fetal Hgb (Kleihauer Betke) |

# CLIA Complexity: High Complexity

# Clinical Utility: Determination of the qualitative volume of fetal blood to have entered the maternal circulation during trauma, invasive procedures, or delivery. In order to ensure Anti-D alloimmunization does not occur in the mother further testing will be performed to determine the dosing of RhIG required for protection against alloimmunization.

# Principle:

The Fetomaternal Hemorrhage screening test is a qualitative test for detection of fetal Rh(D) positive fetal cells in the circulation of Rh(D) negative mothers. A red blood cell suspension from the mother post-delivery of all products of conception will be incubated with monoclonal IgM Anti-D antibodies, washed to remove unbound Anti-D, and indicator cells that are of the R2r phenotype will be added and examined microscopically for the formation of rosettes around Rh(D) positive fetal cells.

# Specimen Collection:

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample Type** | **Container** | **Minimum Volume** | **Stability****Max Storage Temp** |
| Whole Blood collected from the mother preferably >1 hour post delivery | EDTA | 0.5 mL  | 48 hours(1-10°C) |
| Whole Blood collected from the mother that is >20 weeks gestation and has experienced abdominal trauma or miscarriage | EDTA | 0.5 mL | 48 hours(1-10°C) |

# Materials:

|  |  |  |
| --- | --- | --- |
| **Reagents*** Immucor, Inc. FMH RapidScreen Kit
 | **Supplies / Materials*** 12 x 75 mm test tubes
* Isotonic saline (0.9%)
* Glass slides
* Gauze
* Pipettes
 | **Equipment*** Cell Washer
* Calibrated centrifuge
* Microscope
* Timer
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# Quality Control

## Quality Control Material: Positive and negative controls are included in the FMH RapidScreen kit.

## Frequency: Positive and negative controls must be performed with each batch of tests.

# Procedure

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| --- | --- | --- |
| **Step** | **Action** | **Related Documents** |
| 1 | Bring all reagents to room temperature |  |
| 2 | Check the identification on the sample and resolve any discrepancies before proceeding. | 7540.BB.CC.121 Evaluating Patient Samples and Request Forms |
| 3 | Prepare a once washed 2-4% suspension of well mixed maternal red blood cells | 7540.BB.CC.101 3%-5% Red Cell Suspension Preparation |
| 4 | Properly label 12 x 75 mm test tubes for quality control (positive and negative) and patient(s). |  |
| 5  | Add one drop of the Anti-D reagent included in the kit |  |
| 6  | Add one drop of the appropriate well mixed red blood cell suspension to the properly labeled tube. |  |
| 7 | Mix well and incubate for 5 minutes (± 1 minute) at room temperature (18°C - 30°C) |  |
| 8 | Wash cells four times with saline |  |
| 9 | Decant saline completely after the last wash |  |
| 10 | Add one drop of Indicator cells to each tube and mix well |  |
| 11 | Centrifuge at saline setting |  |
| 12 | Gently resuspend cell button |  |
| 13 | Examine under a microscope using low power magnification. Note: Examination under the microscope may be performed in the tube or on a microscope slide. If clumps are seen on the tube, the contents must be transferred to a slide so that number of clumps can be counted. |  |
| 14 | Count at least 5 separate fields and record the total number of rosettes observed. |  |
| 15 | If | Then | 7540.BB.CC.116 Direct Antiglobulin Test (DAT) by Tube Testing |
| ≤ 4 rosettes observed | Test is negative |
| ≥ 5 rosettes observed | Test is positive. A Fetal Hgb stain (Kleihauer-Betke) must be ordered and performed for quantification.Note: Perform a Polyspecific Direct Antiglobulin Test (DAT) to ensure the result is not a false positive. Refer to the Limitations of Procedure section of this procedure. If the DAT is negative, then the result is valid. If the DAT is positive, then result the test as Err and enter a comment stating, “Fetal Screen is invalid due to positive DAT. See Fetal Hgb for result.” |
| 16 | If the fetomaternal hemorrhage screening test has more rosettes than the positive control, then repeat the fetomaternal hemorrhage screening test and perform a Weak D test to verify that the mother is Rh(D) negative and that the test is valid.Note: If the mother is Rh(D) negative, the infant’s Rh(D) type is unknown, and the fetomaternal hemorrhage screening test has more rosettes than the positive control, then order a Fetal Hgb Stain (Kleihauer-Betke) and do not interpret the fetomaternal hemorrhage screening test until the Fetal Hgb Stain is complete. If the Fetal Hgb Stain correlates with the fetomaternal hemorrhage screening test, then report as positive. If the Fetal Hgb Stain does not correlate with the fetal bleed screen, then report as Err and reference the Fetal Hgb Stain results in the comment field. | 7540.BB.CC.113 Weak D Test by Tube Testing |

# Interpretation of Results

## For test results to be valid the negative control must be negative and the positive control must be positive. The test must be repeated when the expected control results are not obtained. Patient results must not be reported if control results are not acceptable.

## If after examining five low power fields, if less than four rosettes are observed (less than one clump per low power field), the test is considered negative, indicating that a significant fetomaternal hemorrhage did not occur.

## The presence of one or more rosettes in five low power filed constitutes a positive test and indicates the presence of Rh(D) positive red blood cells in possibly significant numbers in maternal blood.

### A quantitative test (Kleihauer Betke) must be performed to determine if more than one syringe of RhIG (300 µg) should be administered to the mother.

# Result Reporting

## Reference Range

### Results are negative if after examining five low power fields ≤ 4 rosettes observed

### Results are positive if after examining five low power fields ≥ 5 rosettes observed

# Limitations of Procedure

## Factors such as contaminated materials, improper incubation time and temperature, centrifugation, examination for agglutination, and deviation from the recommended test procedure, may cause false test results.

## In order to correctly interpret test results, testing must be performed on the blood of a known Rh(D) negative mother of a recently delivered Rh(D) positive infant.

### If the infant’s red blood cells possess a Weak D or partial D antigen, then the test may not detect a fetomaternal hemorrhage greater than 30 mL of whole blood. In order to detect a fetomaternal hemorrhage a Fetal Hgb (Kleihauer Betke) test must be performed.

### If the mother is Weak D positive, then the test is invalid and a Fetal Hgb (Kleihauer Betke) test must be performed.

### If the infant is Rh(D) negative, then the test is not indicated and should not be performed.

## A fetomaternal hemorrhage screening test may be performed on Rh(D) negative mothers that are >20 weeks gestation and have experienced abdominal trauma and/or miscarriage. Since the Rh(D) type of the fetus is unknown a positive test must be interpreted with caution. A Fetal Hgb Stain (Kleihauer-Betke) must be ordered on all fetomaternal hemorrhage screening tests that appear positive.

## Failure to carry out the washing stages of the test procedure properly may cause false positive test results due to the agglutination of the indicator cells by the free Anti-D remaining in the test system.

## If there is an ABO incompatibility between the mother and child, the mother’s naturally occurring ABO antibodies may destroy any fetal cells in the mother’s blood sample before testing is performed. This is true for any method of detecting fetal cells in the maternal blood.

## Incomplete and/or inadequate washing process of the test procedure may result in false positive test results, due to agglutination of the indicator cells and the free Anti-D remaining in the test system.

## A false positive test result may occur if the maternal red blood cells have a positive direct antiglobulin test due to an autoantibody reacting with the indicator cells. The FMH RapidScreen would be considered invalid and a quantitative Fetal Hgb test (Kleihauer Betke) must be performed. If the DAT is positive, then result the test as Err and enter a comment stating, “Fetal Screen is invalid due to positive DAT. See Fetal Hgb for result.”

## A positive test result does not provide evidence that an increased dose of RhIG is required to protect the mother from Anti-D alloimmunization, but only indicates that a significant fetomaternal hemorrhage may have occurred. A quantitative procedure such as the Fetal Hgb (Kleihauer Betke) is required to determine the volume of fetomaternal hemorrhage.

## Grossly hemolyzed specimens are not acceptable and should be recollected before testing.

# Definitions

## Fetomaternal hemorrhage: Variable volumes of fetal blood entering into the maternal circulation before or during delivery, which may cause alloimmunization

## Alloimmunization: Immune response due to exposure to foreign antigens, which results in the production of antibodies

## Kleihauer-Betke: Acid elution test that utilizes the resistance of hemoglobin F in order to visualize fetal cells in maternal circulation. The ratio of fetal cells to total cells counted is multiplied by the maternal blood volume and the fetomaternal hemorrhage volume is quantitated. The required dosage of RhIG, in order to prevent Rh(D) alloimmunization is calculated from the fetomaternal hemorrhage volume.

# References:

## Fung, M.K. (Current Edition). *Technical Manual.* Bethesda, MD: AABB.

## *Standards for Blood Banks and Transfusion Services* (Current Edition). Bethesda, MD: AABB.

## Immucor, Inc. (Current Revision). *FMH RapidScreen* [Manufacturer’s Insert.] Norcross, GA.

# Related Documents

## 7540.BB.CC.101 3%-5% Red Cell Suspension Preparation

## 7540.BB.CC.113 Weak D Test by Tube Testing

## 7540.BB.CC.116 Direct Antiglobulin Test (DAT) by Tube Testing

## 7540.BB.CC.121 Evaluating Patient Samples and Request Forms