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| Policy Statement | The laboratory will provide this test to detect fetal bleeds in D (Rho) negative pregnant patients as required in AABB Standards for Blood Banks and Transfusion Services. |
| Purpose | To detect fetal maternal hemorrhage and to determine the correct dosage of Rh Immune Globulin. |
| Scope | Obstetric Patients, D (Rho) Negative who have delivered Rh positive babies. |
| Responsibility | The laboratory responds to requests as generated by the appropriate primary caregiver who identifies patients meeting criteria for testing. |
| Related Documents | * Immucor, Fetal Bleeding Screen package insert.
* AABB Standards for Blood Bank and Transfusion Services, current edition.
* AABB Technical Manual, current edition, Bethesda, MD.
* TRAN 6024 R Cord Hold and Cord Blood Evaluation Procedure
* TRAN 6004 P Weak D Testing Process
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**Principle**

A red blood cell suspension from the D-negative mother is first incubated for **5 minutes at room temperature** with a reagent containing anti-D and then washed to remove all 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 blood 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 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 to the mother.

**Specimen**

1. Collect one 6 ml pink top tube from mother after the delivery of all products of conception, preferably one hour following delivery.
2. Refrigerate at 1° to 10° C if delay in testing occurs. Do not store longer than two days.
3. Do not use if grossly hemolyzed.
4. Do not centrifuge specimen tube.

## Reagents

1. Fetal Bleed Screening Test Kit:
	1. Anti-D serum (chemically modified).
	2. Indicator Cells.
	3. Positive Control.
	4. Negative Control.
2. Test tubes. 12 x 75 mm
3. Pipettes (plastic, disposable blood bank style)
4. Microscope with low power (50X to 100X) capability
5. Microscope slides
6. Test tube Centrifuge.
7. 0.9% saline, preferably phosphate buffered

## Procedure

1. Make a 2-4% suspension of the well-mixed maternal red blood cells to be tested in isotonic saline.
2. Place one drop of the prepared maternal red blood cell suspension in a properly labeled test tube.
3. Add one drop of the Anti-D Reagent. Note: Steps 2 and 3 may be reversed, if desired.
4. Mix well and incubate for 5 minutes (± 1 minute) at room temperature (18°C to 30°C).
5. Wash the red blood cells four times with the tubes filled with saline, being careful to decant the saline completely between washes and to re-suspend the red blood cells thoroughly when adding saline for the next wash. Note: More than four washes may be required if the test is carried out in a smaller test tube than that recommended.
6. Decant the saline completely after the last wash.
7. Add one drop of Indicator Cells and mix well by gently shaking the tube.
8. Centrifuge immediately at a time appropriate to the calibration of the centrifuge.
9. Re-suspend the red blood cell button completely and examine five (5) low-power fields microscopically for mixed-field agglutination using approximately 100× magnification. Note: Microscopic examination can be carried out either in the tube or on a microscope slide. If agglutinates are seen in the tube, the contents should be transferred to a microscope slide so that the number of agglutinates per low-power field can be evaluated.
10. Refer to package insert for additional information.

## Interpretation

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| FETAL BLEED SCREEN |
| Patient  | Positive Control  | Negative Control  | Interpretation |
| Negative – Equal or less than 4 clumps in 5 fields.  | +  | 0 | Significant bleed has not occurred  |
| Positive - Equal or greater than 5 clumps in 5 fields | +  | 0  | Significant bleed has occurred, perform Kleihauer - Betke |
| 0  | +  | +  | invalid, repeat test  |
| +  | +  | +  | invalid, repeat test  |
| 0  | 0  | 0  | invalid, repeat test  |

Code: 0 = negative; + = positive

## Results Reporting

1. All the following information must be concurrently documented in the laboratory information system (see attachments):
	1. Fetal Bleed Screening lot number
	2. Fetal Bleed Screening expiration date
	3. Patient Results
	4. Positive Control Results
	5. Negative Control Results
	6. Interpretation as positive or negative
2. A positive result must be reported to the nurse in charge of the patient.
3. If a strong positive result is noted, the blood type and Rh should be repeated to insure the correct patient’s blood is being tested. A weak D test should be performed if the ABO/Rh type matches. If the mother’s weak D test is positive, a KB stain should be performed with the comment of inaccurate results due to the patient’s weak D status. See TRAN 6004 P Weak D Testing Process.
4. The positive Fetal Bleed Screen test result is reflexed to a Kleihauer-Betke test.
5. Send the specimen to Hematology for a Kleihauer-Betke (KB) test.

## Limitations

As in all serological tests, such factors as contaminated materials, improper incubation time, temperature, centrifugation, examination for agglutination and deviation from the recommended test procedure, may give rise to false test results. In addition:

1. For correct interpretation of the test results, the test must be performed on the blood of a known D-negative mother of a recently delivered D-positive child. If the infant's red blood cells possess a weak D antigen or partial D antigen, the test may not detect a feto-maternal hemorrhage exceeding 30 mL of whole blood. When the D antigen on the infant's red blood cells requires a weak D test for detection, a test to detect feto-maternal hemorrhage based on fetal hemoglobin is recommended. If the mother is D-positive, including weak D, strong agglutination provides no information about the extent of feto-maternal hemorrhage. If the infant is D-negative, a negative test result can be expected to occur, regardless of the volume of feto-maternal hemorrhage.
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 Rh-Immune Globulin 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 red blood cells may tend to diminish over the dating period.
7. Do not use grossly hemolyzed specimens for testing.