**Fetalscreen**

**INTENDED USE:** A qualitative screening test to detect Rh-D positive fetal red blood cells in the maternal circulation.

**PRINCIPLE**: A red blood cell suspension from the D-negative mother is incubated with a serum containing anti-D, and then washed to remove all unbound antibody. A weak suspension of ficin-treated D-positive red cells is added. The cell mixture is centrifuged and examined for mixed-field agglutination (rosette appearance). The indicator cells form clumps around the individual cells of the minor population of D-positive red cells from the baby, leading to the rosette appearance. A positive fetal screen test is 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.

**REAGENTS: (Store at 2-8C)**

1. Fetalscreen II Antibody reagent: a blend of 2 monoclonal anti-D antibodies.

2. Indicator cells: 0.5% suspension of enzyme treated group O rbcs from a donor with an R2R2 phenotype.

3. Positive (Pos) Control: 2-3% suspension of human group O rbcs; approximately 99.4% D-negative cells and

0.6% D-positive cells.

4. Negative (Neg) Control: 2-3% suspension of human Group O, D- negative cells

**SPECIMEN COLLECTION AND PREPARATION:** No special preparation of the patient is required prior to specimen collection. The maternal blood should be collected as soon as possible after deliver, prior to administration of therapeutic anti-D. It is best to wait at least an hour after delivery to allow any fetal blood to mix thereafter. Blood should be collected into EDTA and tested upon collection. If there is a delay in testing, blood should be stored at 2-8C, and tested within 48 hrs. of specimen collection.

**MATERIALS REQUIRED:** 12 x 75 mm test tubes, isotonic saline, pipettes, 37C incubator, timer, centrifuge, microscope.

**PROCEDURE: (Bring all reagents to room temperature before testing)**

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| **Step** | **Action** |
| 1 | Use isotonic saline to make a 2-3% suspension of the maternal red blood cells to be tested. |
| 2 | Label 3 test tubes: 1 each- Patient, Pos control, Neg Control (or 1,2 and 3) |
| 3 | Add one drop of Fetalscreen II Antibody Reagent to each tube. |
| 4 | Patient Tube (1): Add one drop of maternal cell suspension, using clean pipette. |
| 5 | Pos Control Tube (2): Add one drop of Fetalscreen II Positive Control Cells. |
| 6 | Neg Control Tube (3): Add one drop of Fetalscreen II Negative Control Cells. |
| 7 | Mix well and incubate the tubes for 15 + 2 minutes at 37 + 1C. |
| 8 | Using the cell washer: Wash the contents of the tubes 4 times with isotonic saline.  1. If performing a manual wash: Decant the saline completely after each wash, decanting  the last wash thoroughly. |

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| **Step** | **Action** |
| 9 | Add 1 drop of thoroughly mixed Fetalscreen II Indicator Cells to each tube and mix well by gently shaking each tube. |
| 10 | Centrifuge for 15-45 seconds at approx. 3400 rpms. (Extending spin time to 45 seconds may result in an improvement to the size, number and quality of rosettes) |
| 11 | Resuspend the cell button completely. |
| 12 | Examine under a microscope, using low power magnification.  1. Examination can be done in a tube or on a microscope slide. If clumps are seen in the  tube, carefully pour onto a microscope slide for examination and rosette counting. |
| 13 | Count 5 separate fields and record the number of rosettes or agglutinates observed. |

**INTERPRETATION OF RESULTS**

**Positive Test:** Three or more clumps of agglutinated rbcs observed after examining 5 low-power fields.

● This indicates the presence of D-positive fetal rbcs in possibly significant numbers in the maternal blood.

● The number of rosettes observed microscopically does not correlate with the number of positive cells

present in the maternal blood sample The test merely provides evidence that a potentially large feto-

maternal bleed may have occurred.

● A quantitative test is required to determine the volume of feto-maternal hemorrhage as a means of

estimating the dosage of Rh-Immune Globulin needed to prevent Rh immunization.

**Negative Test:** Two or less clumps of agglutinated rbcs observed in 5 low-power fields.

● This indicates that a large feto-maternal hemorrhage did not occur.

● A negative test indicates that a < 30 ml bleed has occurred and that one dose of Rh Immune Globulin

is sufficient.

**QUALITY CONTROL:** For a test result to be considered valid, the results of the FETALSCREEN II Negative Control Cells run at the same time must be negative and the results obtained with the FETALSCREEN II Positive Control Cells run at the same time must be positive. Tests are invalid and must be repeated if the proper control results are not observed.

**LIMITATIONS:**

1. The test must be performed on a D-negative mother of a recently delivered D-positive child. If the

infant’s rbcs possess a weak D antigen, the test may not detect a feto-maternal hemorrhage exceeding

30 ml of whole blood.

2. Performing this test on a Weak D (Du) positive mother will result in a false positive result.

3. For additional limitations, refer to Instructions for Use product insert.

**REFERENCES:**

Roback, J. (2011). Technical manual (17th ed.). Bethesda, Md.: AABB.

Fetalscreen II Instructions for Use: Pub No. J32984\_EN: Version 1.0 07/01/2009

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