FMH RapidScreen

For the Detection of D-Positive Red Cells in D-Negative Mothers

IVD | Rx ONLY | 1°C–10°C | Do not freeze

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER. DO NOT PIPETTE THIS PRODUCT BY MOUTH. AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.

Kit Components: ●**Anti-D Reagent** Harmful, Preservative: 0.1% Sodium Azide
Do not use if markedly turbid. No US standard of potency.
●**Indicator Cells** ●**Positive Control** CONTROL + ●**Negative Control** CONTROL –
Do not use if markedly hemolyzed.
Red blood cell preservatives: neomycin sulfate (0.1 mg/ml), chloramphenicol (0.25 mg/ml) and gentamycin sulfate (0.05 mg/ml)

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3047-3

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IMMUCOR

Intended Use:
FMH RapidScreen is intended for use in the detection of D-positive red blood cells in D-negative mothers.

Summary of the Test:
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. In susceptible subjects, this event may give rise to the production of maternal alloantibodies directed at antigens present on the erythrocytes of the fetus but absent from those of the mother. In most instances, 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. Pollack and his associates calculated that the standard 300 µg dose of Rh-Immune Globulin is sufficient to suppress Rh immunization providing no more than 15 mL of fetal red blood cells (equivalent to 30 mL of whole blood) have entered the maternal circulation.[1] Thus, several laboratory procedures have been applied to quantitate the volume of feto-maternal hemorrhage in post-partum D-negative women, as a means to determine when greater than the standard 300 µg dose of Rh-Immune Globulin is required to afford protection. A test to detect a feto-maternal hemorrhage of an amount greater than that covered by the standard 300 µg dose of Rh-Immune Globulin is a requirement of AABB Standards.[2]

Methods based on the acid-elution method of Kleihauer, Braun and Betke utilize the resistance of fetal hemoglobin (hemoglobin F) to elute into an acid buffer, and enable fetal red blood cells to be recognized and counted by microscopic examination.[3] To overcome inherent disadvantages of the method, two serological techniques based on an enzyme-linked immunoabsorbant assay (ELISA) procedure have been proposed as a more objective means of detecting and quantitating small percentages of D-positive red blood cells in D-negative blood.[4,5]

To minimize the need to carry out a quantitative procedure in all cases, a sensitive serological screening test has been proposed by Sebring and Polesky, which applies the principle of immune rosetting.[6,7]

Principle of the Test:
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.

Reagents:
- **Anti-D Reagent:** Contains monoclonal IgM anti-D antibodies from the human/murine heterohybridoma[8] cell line GAMA401 grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Any Bovine Albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have a low-TSE (Transmissible Spongiform Encephalopathy) risk. Contains 0.1% sodium azide as a preservative.
- **Indicator Cells:** An approximate 0.5% suspension of group O red blood cells obtained from a donor of the DcEe (R_2r) phenotype.
- **Positive Control:** A 2-4% suspension of red blood cells comprising approximately 99.4% of group O D-negative cells and approximately 0.6% group O D-positive cells obtained from a donor having heterozygous expression of the D antigen.
- **Negative Control:** A 2-4% suspension of group O D-negative red blood cells.

Key:
Underline = Addition or significant change ▲ = Deletion of text

All red blood cells are washed to remove blood group antibodies and are resuspended in a buffered solution to which neomycin sulfate (0.1 mg/ml), chloramphenicol (0.25 mg/ml) and gentamycin sulfate (0.05 mg/ml) have been added as preservatives. The components of the kit may be interchanged between lots, providing they are in date.

Precautions:
For in-vitro use. No US standard of potency. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use beyond the expiration date. Marked hemolysis and/or darkening of the cells are indication of product deterioration. Effort should be made to minimize contamination and prevent evaporation during use of the product. Gently resuspend cell suspensions before using. The Indicator Cells must be well mixed before use.

CAUTION: Do not pipette this antisera product by mouth as the absence of murine virus has not been determined.

The Anti-D Reagent must not be used to type red blood cells for the D antigen. Do not use if markedly turbid.

Anti-D Reagent contains 0.1% sodium azide. Warning: H302 Harmful if swallowed. Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.
THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Handle and dispose of reagent as if potentially infectious.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

Specimen Collection and Preparation:
No special preparation of the patient is required prior to specimen collection. The test procedure requires a blood specimen collected from the MOTHER after delivery of all products of conception. It is best to wait about an hour after delivery to allow any fetal blood to mix thoroughly in the maternal circulation, but the sample should be collected as soon as possible thereafter.[9] Blood should be drawn by an aseptic technique into EDTA. If a delay in testing should occur, the specimen must be stored at 1°C to 10°C. Bacterial contamination of the specimen may cause false test results. Blood drawn into EDTA should not be stored for longer than two days, but the test must be carried out as soon as possible in any event, in order to permit the administration of Rh-Immune Globulin within 72 hours of delivery. Do not use grossly hemolyzed specimens for testing.

Procedure:
Materials Provided:
FMH RapidScreen.
Additional Materials Required:
1. Test tubes (12×75 mm are recommended).
2. Pipettes.
3. Microscope slides.
4. Isotonic saline, preferably Phosphate-buffered saline, (approximately 15 mM), pH 6.5-7.5
5. Timer.
6. Centrifuge.
7. Microscope.

Test Method:

1. Make a 2-4% suspension of the well-mixed maternal red blood cells to be tested in isotonic saline.
2. Place 1 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 resuspend 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 for:
   (a) 15 seconds at 3400rpm (RCF 900 to 1000) or
   (b) a time appropriate to the calibration of the centrifuge.
9. Resuspend 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.

Stability of the Reaction:

Test and control results should be interpreted immediately upon completion of the test.

Quality Control:

In parallel with each batch of tests (or with each test if performed singly), it is recommended that the entire test procedure be performed on both the positive and the negative control cells supplied.

The positive control test with the supplied mixture of D-negative and D-positive red blood cells confirms the reactivity of the indicator cells and provides an indication that the test is being carried out correctly. The positive control must demonstrate a positive reaction (five or more agglutinates per five low-power fields). The negative control test is needed to assure that the washing procedure employed has been sufficient to remove all unbound anti-D. The presence of agglutinates in the negative control test would suggest that the indicator cells are being agglutinated by unbound anti-D remaining in the tube and may indicate that the current wash procedure is not sufficient and additional wash steps are needed. To be valid, the negative control test must demonstrate a negative reaction (four or fewer agglutinates per five low-power fields).

Interpretation of Results:

Positive test: After examining five low-power fields, if five or more agglutinates of red blood cells are observed, the test is positive and indicates the presence of D-positive fetal red blood cells in possibly significant numbers in the maternal blood.

Negative test: After examining five low-power fields, if four or fewer agglutinates of red blood cells are observed, the test is negative, indicating that a large feto-maternal hemorrhage did not occur.

The number of agglutinates observed may be influenced by several variables in the performance of the test procedure and should not therefore be used as a means of quantitating the amount of feto-maternal hemorrhage. A positive test merely provides evidence that a potentially large feto-maternal bleed might 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. Authors cited by Mollison, Engelfriet and Contreras have estimated that as many as 0.3% of recently delivered women may be expected to have 10 mL or more of fetal red blood cells in their circulation, and that 1% of women may be expected to have 3 mL or more at the time of delivery.[10] Caesarean section and manual removal of the placenta are associated with a significant increase in the number of fetal cells finding their way into the maternal circulation.

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.

Specific Performance Characteristics:

FMH RapidScreen, if carried out strictly in accordance with the recommended test procedure, will detect feto-maternal hemorrhage whenever 30 mL or more of ABO-compatible D-positive fetal blood has entered the maternal circulation. Depending on the care taken in carrying out the test, a positive result may be obtained when the extent of feto-maternal bleeding is less than 30 mL, but the test is designed to give a negative result when, as in most cases, the amount of fetal bleeding is small (e.g. less than 2 mL of whole blood). All red blood cell suspensions are tested and determined to give a negative direct antiglobulin test.

The performance of FMH RapidScreen was compared to Fetal Bleed Screening Test on 547 samples (186 contrived samples and 361 clinical samples). The results are summarized in the following table.

		Fetal Bleed Screening Test		
		Pos	Neg	Total
FMH RapidScreen	Pos	150	1	151
	Neg	9	387	396
	Total	159	388	547

Positive % Agreement = 94.3% (One-sided 95% lower confidence limit = 90.3%)

Negative % Agreement = 99.7% (One-sided 95% lower confidence limit = 98.8%)

Overall % Agreement = 98.2% (One-sided 95% lower confidence limit = 96.9%)

A subgroup analysis of the above clinical data was performed for 130 contrived samples containing a feto-maternal bleed of 20 mL or greater and compared the performance of FMH RapidScreen to Fetal Bleed Screening Test. The results are summarized in the following table.

		Fetal Bleed Screening Test		
		Pos	Neg	Total
FMH RapidScreen	Pos	126	0	126
	Neg	1	3	4
	Total	127	3	130

Positive % Agreement = 99.2% (One-sided 95% lower confidence limit = 96.5%)

The performance of this product is dependent on adhering to the recommended methods found in this insert. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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8. Thompson KM, Melamed MD, Eagle K, et al. Production of human IgG and IgM antibodies with anti-D (rhesus) specificity using heterohybridomas. Immunology 1986; 58:157-160.
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