UnityPoint Health Pekin
Department of Pathology
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Effective Date: 11/29/18
Date Reviewed: 10/23/18, 11/29/18
TRM.40790, TRM.40800

FMH RAPID SCREEN

Test Code: FS and RHIGP

I. 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 case 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.

II. CLINICAL SIGNIFICANCE

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. 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.

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 resetting.

A fetal screen will be performed immediately following delivery of Rh positive baby born to Rh-negative mother or whenever there is the possibility that fetal blood may enter maternal circulation (i.e., spontaneous abortion, invasive obstetric procedures, and abdominal trauma, during amniocentesis).

III. SPECIMEN

A. Blood should be collected from the mother in EDTA (lavender or pink EDTA acceptable) 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.
B. Store anticoagulated whole blood specimen at 1° to 10° C until tested.
C. Blood should not be stored for longer than 2 days.
D. Do not use grossly hemolyzed specimens for testing.

IV. REAGENT

A. Anti-D Reagent: Contains monoclonal IgM anti-D antibodies from the human/murine heterohybridoma 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.
B. Indicator Cells: An approximate 0.5% suspension of group O red blood cells obtained from a donor of the DcEe (R_2r) phenotype.
C. 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.
D. Negative Control: A 2-4% suspension of group O D-negative red blood cells.
    1. 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 gentamicin sulfate (0.05 mg/ml) have been added as preservatives.
    2. 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.

V. INSTRUMENTATION/EQUIPMENT

A. 12 x 75 mm Test tubes
B. Disposable Plastic pipettes
C. Microscope slides
D. Timer
E. Centrifuge
F. Microscope

VI. QUALITY CONTROL

A. In parallel with each batch of tests (or with each test if performed singly), the entire test procedure will be performed on both the positive and the negative control cells supplied in the FMH Rapid Screen kit.

VII. PROCEDURE

A. Prepare a 2-4% suspension of washed maternal red blood cells in saline.
B. Label three 12 x 75 mm test tubes, +, - and PT
C. Label each tube with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate patients with the same initials, if necessary.)
D. To tube +, add one drop of the fetal screen positive control cells.
E. To tube -, add one drop of fetal screen negative control cells.
F. To tube PT, using a clean Pasteur pipette, add one drop of the maternal cell suspension.
G. To each of the three tubes, add one drop of the Anti-D serum.
H. Mix well and incubate the tubes for 5 minutes at room temperature (18° to 30° C)/
I. Wash the contents of the tubes 4 times with saline. Decant the last wash thoroughly (an automatic cell washer may be used).
J. Add one drop of fetal screen indicator cells to each tube.
K. Mix well.
L. Centrifuge the tubes for 15 seconds, or at the optimum calibrated spin time, at 3400 rpm.
M. Resuspend the red blood cell button completely and examine five (5) low-power fields microscopically for mixed-field agglutination using approximately 100x magnification.
N. Reagent Control Check will be done on new lot number of fetal screen kits. Use old lot number controls with new lot number reagents and document on the back page of the Daily Quality Control log.

VIII. REPORTING RESULTS

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

B. Positive test:
   1. After examining five low-power fields, if five or more agglutinates of red blood cells are observed, total in all of the five fields, the test is positive and indicates the presence of D-positive fetal red blood cells in possibly significant numbers in the maternal blood.
      a. All positive specimens are to be sent to ARUP for a Fetal Hemoglobin test (ARUP #2001743). *Epic Code: LAB762 / Sunquest Code: FETHGA* . They will accept a pink or lavender whole blood EDTA tube, minimum 0.5ml, refrigerated for up to 72 hours.
      b. If we receive one on a Friday after ARUP's last pick-up until Sunday we need to call Arup on Saturday morning for a STAT pickup (for an additional charge). Call the Client Service # 800-522-2787 and give them our Client ID# 323184.

C. Negative test:
   1. After examining five low-power fields, if four or fewer agglutinates of red blood cells, total in all 5 fields, are observed, the test is negative, indicating that a large feto-maternal hemorrhage did not occur.

D. Report reactions of positive control, negative control, and the patient, along with a Positive or Negative Interpretation in Sun Quest- Blood Order Processing (BOP) immediately after reading reactions.

E. Record QC results on the back side of the Reagent Quality Control Log.

IX. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. As in all serological tests, such factors as contaminated materials, improper incubation times, 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 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.

X. REFERENCES

A. Immucor, Inc., Norcross, GA, FMH Rapid Screen, 3047-3, Rev 3/2017.

POLICY CREATION :		***Date***
Author:	***Sharrol Brisbin, MT (ASCP)***	***11/01/2004***
Medical Director:	***Kathryn Kramer, MD***	***11/01/2004***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
12-5-18	Kathryn Kramer MD	
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date
11/29/18	Positive specimens sent to ARUP	Jenny Turner	11/29/18

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
Jennifer Turner	12-3-18				