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Beaumont

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Area	Laboratory-Chemistry
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APT (Alkali Denaturation) Test for Fetal Hemoglobin - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A. The APT test (Alkali Denaturation) is used to distinguish between maternal and infant's blood. It is based on the fact that the hemoglobin in an adult (Hemoglobin A) differs from the predominant type of hemoglobin in the blood of the fetus and newborn infant (Hemoglobin F). A pink hemoglobin solution prepared from an adult's blood changes to brown-yellow in one to two minutes after the addition of an alkali, because of the conversion of oxyhemoglobin to alkaline globin hematin. If the hemoglobin is primarily fetal hemoglobin, the pink solution is more resistant to denaturation with alkali and retains its pink color.

II. SPECIMEN COLLECTION AND HANDLING:

A. Stool or gastric contents may be used. It is important that the specimen be grossly bloody (red) and not black/tar colored. The test should be performed immediately upon receipt.

B. A specimen which does not show visual evidence of gross blood (not red) is inappropriate for analysis, and the Apt test request should be canceled.

III. REAGENTS:

Prepare 0.25N Sodium Hydroxide (NaOH) fresh each time the test is ordered. Add 250 µL of 10N NaOH to 10 mL of deionized water (DI H_2O) in a sterile orange top urine cup.

IV. QUALITY CONTROL (QC):

A. **To be run with every sample.**

1. **Fetal Hemoglobin Positive:** Obtain an expiring **cord blood (EDTA) specimen** from Blood Bank. Mix the whole blood specimen well. Obtain a stool specimen that is not visibly

bloody. Mix cord blood into stool until the sample is visibly red and bloody.

2. **Fetal Hgb Negative:** Obtain an **adult (EDTA) specimen**. Mix the whole blood specimen well. Obtain a stool specimen that is not visibly bloody. Mix adult whole blood into stool until the sample is visibly red and bloody.
3. **Note**: If a stool specimen is unavailable to prepare QC material, contact microbiology for a stool sample. Make sure to indicate that the stool needs to have no visible evidence of blood.

V. SPECIAL SAFETY PRECAUTIONS:

Adhere to Standard Precautions when handling all specimens. Handle all chemicals in accordance with Beaumont Health Chemical Hygiene Plan and Material Specific Material Safety Data Sheets (MSDS).

VI. PROCEDURE:

A. Assess patient specimen for acceptability.
   1. **Sample must be grossly bloody and RED.** Hemoglobin in black/tar colored specimens has been acted upon by digestive enzymes and is not suitable for testing.
   2. Specimen must be **less than 1 hour old.**

B. Prepare 0.25N NaOH as described above in the Reagents section of the procedure.

C. Label 6, 8 mL conical urine tubes as follows:
   1. Positive Apt control.
   2. Negative Apt control.
   3. Patient last name and order number.
   4. Positive Apt supernatant.
   5. Negative Apt supernatant.
   6. Patient last name and order number supernatant.

D. Hemolyze erythrocytes by mixing with DI H_{20}, ensuring that at least 5 mL of supernatant can be obtained upon centrifugation. (See Quality Control section for QC preparation instructions)
   1. Add approximately 1mL of patient sample and 7 mL of DI H_{20} to the tube labeled with patient last name and order number.
   2. Add approximately 1 mL of positive QC (stool with cord blood) and 7 mL of DI H_{20} into the tube labeled positive Apt control.
   3. Add approximately 1 mL negative QC (stool with adult blood) and 7 mL of DI H_{20} to the tube labeled negative Apt control.

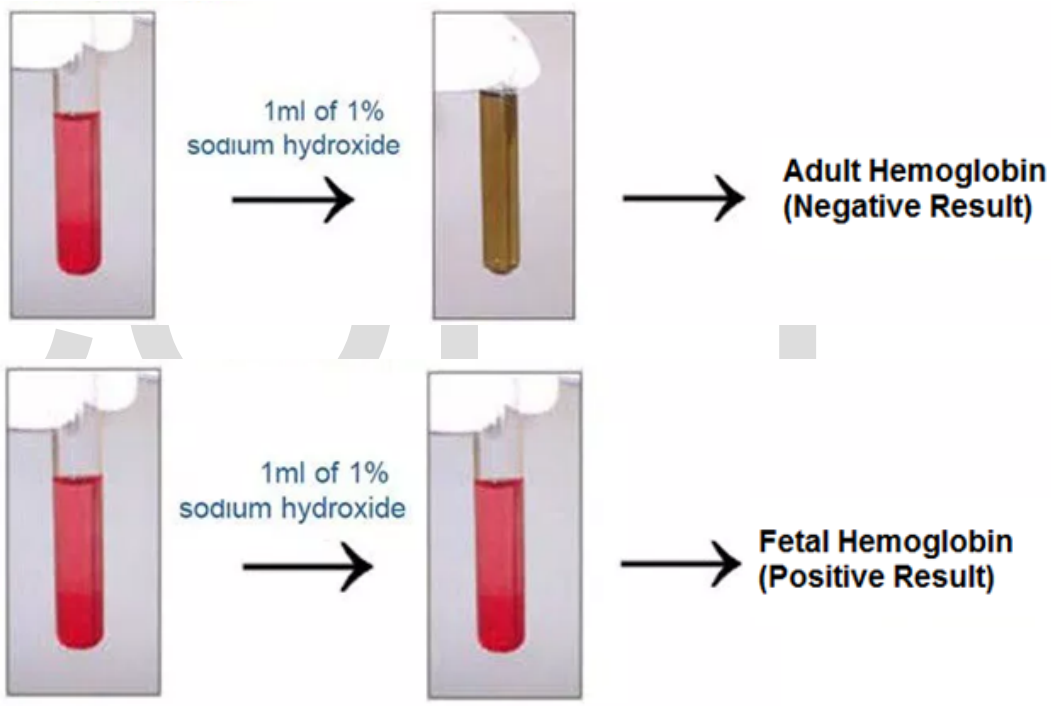
E. Mix all three tubes thoroughly by inversion to ensure complete red cell hemolysis. It may be necessary to shake the tubes vigorously depending on the viscosity of the stool to ensure that red cell hemolysis occurs.

F. Centrifuge at 3500 rpm for 5 minutes and pipette 5 mL of supernatant into the corresponding pre-labelled conical tubes – supernatant must be red/pink.
   1. **Discontinue the procedure if step #5 is not red/pink. Cancel the APT test for fetal Hgb**

with the cancellation reason, "*The Apt test is a qualitative procedure used to visually detect the pink color of Fetal hemoglobin present in a stool or gastric specimen. A specimen which does not show visual evidence of gross blood (pink red) upon receipt by the lab is inappropriate for analysis*."

G. Add 1 mL 0.25 N NaOH to:
    1. 5 mL supernatant of patient sample.
    2. 5 mL supernatant positive control. (contains hemolyzed cord blood).
    3. 5 mL supernatant negative control. (contains hemolyzed adult blood).

H. Observe color of supernatant from step #6 during the following 2 minutes. Maternal Hgb A will denature and change from red/pink to yellow/brown within 2 minutes. **Any red/pink color remaining is evidence for the presence of fetal hemoglobin (positive result)**.
    1. See images below for reference. (Note that these images are of lysed whole blood with no stool present. Use these as a guide to aid in your interpretation.)



VII. INTERPRETATIONS:

A. Adult hemoglobin changes to brown or yellow whereas fetal hemoglobin stays predominantly red/pink. Keep in mind that the solution is being diluted with the addition of the 0.25N NaOH, and a positive result will still become paler when the solutions are mixed. Any red/pink coloring remaining is evidence for a positive result. Negative results will become darker and no red/pink coloring should be left in the solution after 2 minutes.

B. If QC is questionable when running the test, repeat all three tubes (positive, negative, and patient). Enough 0.25N NaOH is made when preparing the reagent to run the test three times.

VIII. REPORTABLE RANGE:

A. Clinical Reportable Range (CRR) = Positive or Negative

B. Report by visualization after alkali denaturation: Fetal Hgb: Negative (or Positive)

IX. LIMITATIONS/INTERFERING SUBSTANCES:

Because this test relies on visual inspection, it is not regarded as highly sensitive at detecting small amounts of Fetal Hemoglobin in the sample.

X. REFERENCES:

1. Apt and Downy, "Melena" Neonatorum: The Swallowed Blood Syndrome. Journal of Pediatrics. 1955, 47:6-12.

Approval Signatures

Step Description	Approver	Date
Medical Director	Ann Marie Blenc: System Med Dir, Hematopath	12/20/2022
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Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	12/15/2022
Lab Chemistry Best Practice Committee	Caitlin Schein: Staff Physician	12/14/2022
Lab Chemistry Best Practice Committee	Qian Sun: Tech Dir, Clin Chemistry, Path	12/9/2022
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