Beaumont Laboratory
Clinical Pathology
Royal Oak, MI 48073

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Related Documents:

APT TEST FOR FETAL HEMOGLOBIN

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Principle

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 (Hgb A) differs from the predominant type of hemoglobin in the blood of the fetus and newborn infant (Hgb 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.

Specimen Collection and Handling

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

Reagents

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

Quality Control

To be run with every sample.

Fetal Hgb Positive: Obtain an expiring **cord blood (EDTA) specimen** from Blood Bank. Mix the whole blood specimen well. Obtain a completed occult blood specimen that is not visibly bloody. Mix cord blood into stool until the sample is visibly red and bloody.

Fetal Hgb Negative: Obtain an **adult (EDTA) specimen.** Mix the whole blood specimen well. Obtain a completed occult blood specimen that is not visibly bloody. Mix adult whole blood into stool until the sample is visibly red and bloody.

Note: If an occult blood 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.

Special Safety Precautions

Adhere to Standard Precautions when handling all specimens. Handle all chemicals in accordance with WBH Chemical Hygiene Plan and Material Specific MSDS.

Procedure

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

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

3. Label 6, 8mL conical urine tubes as follows:
    a. Positive Apt control
    b. Negative Apt control
    c. Patient last name and order number
    d. Positive Apt supernatant
    e. Negative Apt supernatant
    f. Patient last name and order number supernatant

4. Hemolyze erythrocytes by mixing with DI H2O, ensuring that at least 5mL of supernatant can be obtained upon centrifugation. (<u>See Quality Control section for QC preparation instructions)</u>
    a. Add approximately 1mL of patient sample and 7mL of DI H2O to the tube labelled with patient last name and order number
    b. Add approximately 1 mL of positive QC (stool with cord blood) and 7mL of DI H2O into the tube labelled positive Apt control
    c. Add approximately 1mL negative QC (stool with adult blood) and 7mL of DI H2O to the tube labelled negative Apt control

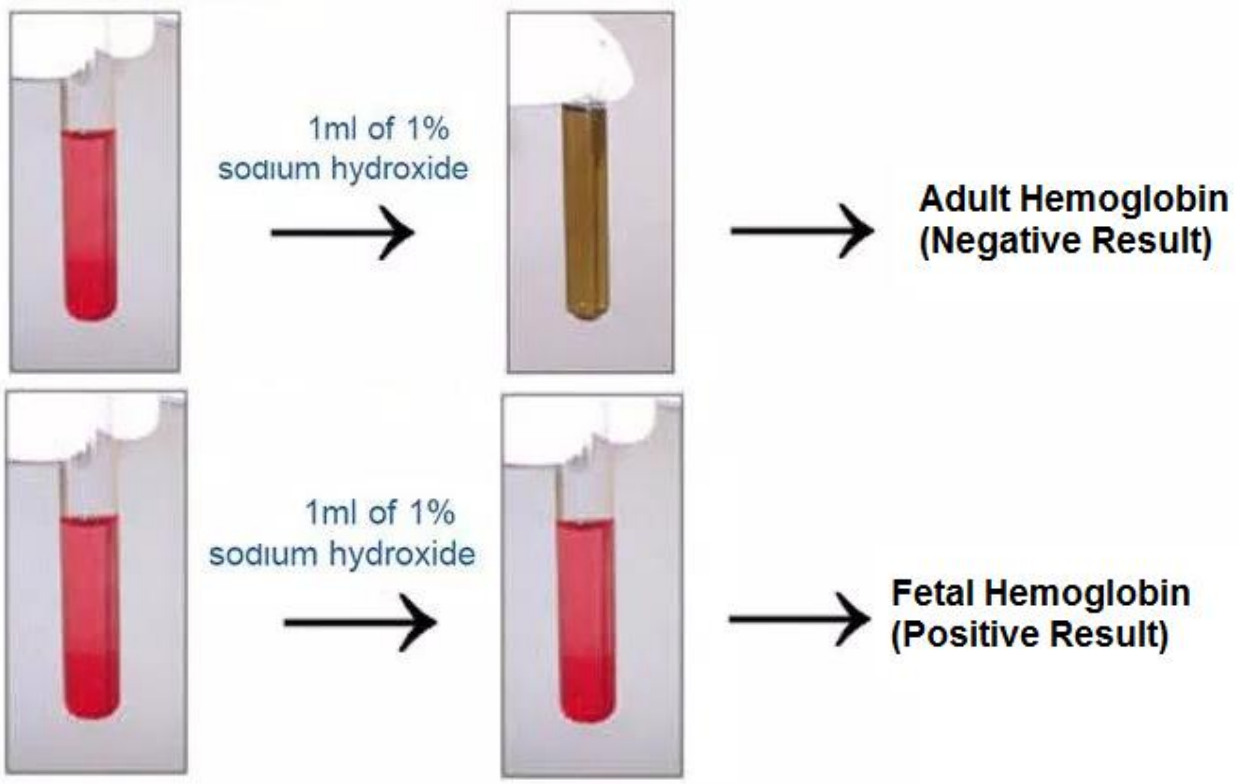
5. Mix all three tubes thoroughly by inversion to ensure complete red cell hemolysis. It may be necessary to shake the tubes vigorously depending on the viscosity of the stool to ensure that red cell hemolysis occurs.

6. Centrifuge at 3500rpm for 5 minutes and pipette 5mL of supernatant into the corresponding pre-labelled conical tubes – supernatant must be red/pink.
    a. **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."*

7. Add 1 mL 0.25 N NaOH to:
    a. 5mL supernatant of patient sample.
    b. 5mL supernatant positive control. (contains hemolyzed cord blood)

c. 5mL supernatant negative control. (contains hemolyzed adult blood)

8. 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)**.
    a. 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.)



Interpretation

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.

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.

Reportable Range

(CRR) Clinical Reportable Range = Positive or Negative

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

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.

References

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

Authorized Reviewers

Section Medical or Technical Director

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Document Control

Location of Master: Master electronic file stored on the Beaumont Laboratory server under S:/AutoChemistry/DocumentControl/NEW/UA/MT/MasterDocuments
Master printed document stored in the Front Desk Procedure Manual, urinalysis section.
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: V. Peterson, MT(ASCP)SC	12/08/2005			
Approved by: R. Karcher, PhD	12/19/2005			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
R. Karcher, PhD	12/20/2006			
R. Karcher, PhD	12/13/2007			
R. Karcher, PhD	01/25/2008	r01	New document control format	
R. Karcher, PhD	12/04/2008			
R. Karcher, PhD	11/10/2009			
Dr. Elizabeth Sykes	01/04/2011			
Elizabeth Sykes, MD	04/26/2012			
Elizabeth Sykes, MD	04/21/2014			
Elizabeth Sykes, MD	11/05/2015			
Elizabeth Sykes, MD	04/05/2018	r02	Updated QC and procedure	
			Images added for reference	
Elizabeth Sykes, MD	05/09/2019	r03	Emphasis of key points	
			Sample dilution altered	