Beaumont Laboratory
Clinical Pathology
Royal Oak, MI 48073

Effective Date:
Supersedes: 04/05/2018
Related Documents:

APT TEST FOR FETAL HEMOGLOBIN

RC.CH.UA.MT.PR.025r02

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 (1%) 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. Add 1mL of cord blood to a completed occult blood specimen that is not visibly bloody. Mix well.

Fetal Hgb Negative: Obtain an **adult (EDTA) specimen.** Mix the whole blood specimen well. Add 1 mL of adult blood to a completed occult blood specimen that is not visibly bloody. Mix well.

Note: If a 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.

APT TEST FOR FETAL HEMOGLOBIN

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 acceptable for testing.)
    b. Specimen must be **less than 1 hour old.**

2. Label 6 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

3. Hemolyze erythrocytes by mixing with DI H2O, ensuring that at least 5mL of supernatant can be obtained upon centrifugation.
    a. Mix 1 part patient sample with 10 parts DI H2O in tube labelled with patient last name and order number
    b. Mix 1 part positive QC (stool with cord blood) with 10 parts DI H2O in tube labelled positive Apt control.
    c. Mix 1 part negative QC (stool with adult blood) with 10 parts DI H2O in tube labelled negative Apt control.

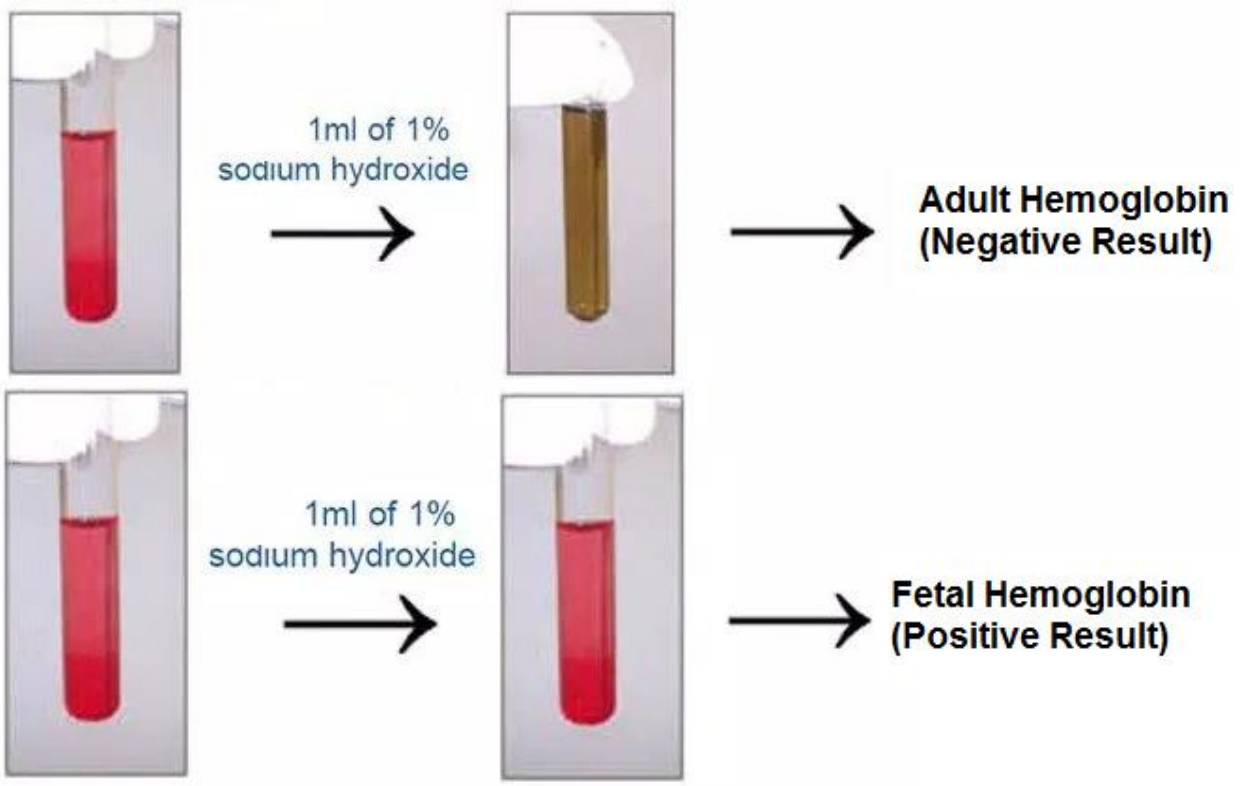
4. Mix all three tubes thoroughly by inversion to ensure red cell hemolysis. It may be necessary to shake the tubes vigorously depending on the viscosity of the stool.

5. 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."*

6. Add 1 mL 0.25 N NaOH (1%) to:
    a. 5mL supernatant of patient sample
    b. 5mL supernatant positive control (hemolyzed cord blood)
    c. 5mL supernatant negative control (hemolyzed adult blood)

7. Observe color of supernatant from step #6 after 2 minutes. Any red/pink color is evidence for the presence of fetal hemoglobin (positive). Negative specimens will change from red/pink to a yellow/brown color.

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 1% 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 1% NaOH is made when preparing the reagent to run the test three times.

Reportable Range

(AMR) Analytical Measurement Range = Positive or Negative
(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 of the sample, it is not regarded as being very sensitive at detecting small amounts of fetal hemoglobin.

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

APT TEST FOR FETAL HEMOGLOBIN

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			
	04/05/2018	r02	Updated QC and procedure	
			Images added for reference	