Beaumont HEALTH

Beaumont Laboratory
Royal Oak

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HEMOSIDERIN IN URINE SEDIMENT

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Principle

Hemosiderin appears in the urine sediment in diseases involving a true siderosis of kidney parenchyma (hemochromatosis). It is also present 2-3 days after an acute hemolytic episode that caused hemoglobinemia and hemoglobinuria. Hemosiderin granules are found in intact renal tubular epithelial cells or occasionally in casts and may also be seen extracellularly. This "dry" procedure employs cytocentrifuge smears of urine sediment and the Prussian-Blue reaction to demonstrate iron in hemosiderin (see Note 1).

Specimen Collection and Handling

Type:	Fresh, random urine
Amount:	Minimum sample size is 10 mL Optimum sample size is 50 mL
Special Handling:	Specimen must be well mixed before initial centrifugation. Specimen must be handled promptly.
Timing:	Specimen is stable for one hour at room temperature; three hours at 4^{o}C.
Criteria for Unacceptable Specimens:	Specimens with low cell counts or volume may give unsatisfactory results.

Supplies

Reagents:

1. 22% Albumin (Sigma Aldrich A-7034)
2. 100% Methanol (Mallinckrodt 3016-4) - (See Note 2.)
3. Iron-free (deionized) water
4. 10% Hydrochloric Acid:
   a. Add 50 mL of concentrated HCl (from Chemistry) to 450 mL distilled water.
   b. Store at room temperature in a large glass bottle.
   c. Stable for one year.
5. Nuclear Fast Red Solution Counterstain
   a. Ready to use. No reconstitution necessary.
   b. Store at room temperature until manufacturer's expiration date on bottle.
6. Prussian-Blue Stain Solution (5% potassium ferrocyanide):
   a. Add 25 mL of deionized or distilled water to a Coplin jar.
   b. Measure out 25 mL of 10% HCl into a graduated cylinder and add approximately 5 mL to a vial of preweighed 2.5 gm potassium ferrocyanide (Sigma P-9387).

CAUTION: Avoid inhaling potassium ferrocyanide.

c. When the potassium ferrocyanide is dissolved, add it to the water in the Coplin jar. Add the remaining HCl to the Coplin jar also.
d. Mix well. Solution should have a yellow color.
e. Prepare **FRESH DAILY**.

Equipment:

1. 15 mL disposable conical centrifuge tubes (Falcon 2905)
2. Centrifuge (IEC)
3. Cytocentrifuge (Shandon)
4. Glass slides
5. Coplin jars
6. Dispo transfer pipets and bulb
7. Cytospin chambers and absorbent blotter cards
8. Wax pencil

Quality Control

1. Run a control slide (patient with known siderocytes).

2. Record the date, control slide ID# and staining results of the control slide in the Cytochemistry Stain QC Book noting any problems or corrections.

3. When preparing control slides, label with (Soft) order number for identification.

4. Keep control slides at room temperature in slide boxes stored in drawers in the bone marrow area.

5. Store stained control smears in “Iron Controls” slide drawer for a minimum of three (3) years.

Procedure

1. Pour approximately 10 mL of urine into a conical centrifuge tube.
2. Centrifuge for 5-10 minutes at 2,000 rpm.
3. Decant supernatant into sink.
4. Re-suspend pellet.
5. Prepare two cytospin smears as follows:
   a. Add 1-2 drops of well-mixed sediment to each cytospin chamber.

   NOTE: When the urine specimen is very cloudy, 1 or 2 drops of un-spun urine can be used instead to avoid overly thick smears.

   b. Add 1 drop of 22% albumin.
   c. Centrifuge chambers for 5 minutes at 1,000 rpm.
   d. **Immediately** after cytocentrifuge stops, remove slides and air dry.
6. Fix smears in formalin-ethanol for 10 minutes (or 100% methanol, See Note 2.)
7. While slides are fixing, prepare stain solution.
8. Wash slides in iron-free (deionized or distilled) water thoroughly 5-6 times.

9. Immerse slides in stain solution for one hour.
10. Rinse in distilled water thoroughly.
11. Counterstain in Nuclear Fast Red Solution Counterstain for 60 seconds.
12. Rinse thoroughly 5-6 times in water.
13. Air dry slides in an upright (standing) position.
    NOTE: Slides may be cover-slipped if desired.
14. Examine thoroughly at 20x, 50x, and 100x oil immersion.

Expected Values

Hemosiderin granules appear as 1-3 micron blue-green granules, singly or in groups, in renal tubular epithelial cells, occasionally in casts, any may even be seen extracellularly. (See Figure 1.)

Results are reported as positive or negative.

Positive urine hemosiderin. Distinct blue granules are apparent within the renal tubular epithelial cells.

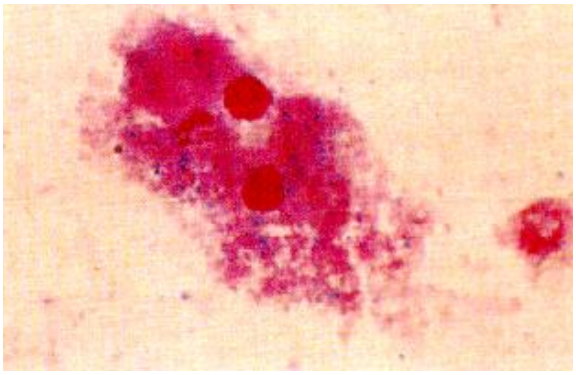

Figure 1

Notes

1. This procedure allows the convenience of staining the urine slides with Prussian Blue along with any bone marrow smears.
2. Although the reference procedures recommend the use of 100% methanol as the fixative, we found the formalin-ethanol fixative used for bone marrow smears to yield comparable results.
3. Hemosiderin is typically found in urine of acid and neutral pH, but not alkaline pH.
4. This technique offers numerous advantages over the "wet" techniques:
   a. The procedure results in slides of uniform quality.
   b. The viewing area is small and readily located.
   c. Cellular morphology is well maintained and highlighted by the counterstain.
   d. The fixed slides are permanent and may be stored for later review.

References

1. Cartwright, G. Diagnostic laboratory hematology. 4th Ed. New York: Gruce & Stratton, 1968:161, 332-333.

2. Henry, JB. Clinical diagnosis and management by laboratory methods. 18th Ed. Philadelphia: WB Saunders Co, 1991:412-413, 429.
3. Ritz, L and Kruskall, MS. An improved urine hemosiderin procedure using cytocentrifugation. Laboratory Medicine 1986;17(5):286.

Attachments

Attachment A – AN IMPROVED URINE HEMOSIDERIN PROCEDURE USING CYTO-CENTRIFUGATION (published article)

Authorized Reviewers

Medical Director, Hematology

Attachment A:

An Improved Urine Hemosiderin Procedure Using Cytocentrifugation

Lori Ritz, MT(ASCP)SH, and Margot S. Kruskall, MD

The presence of hemosiderin granules in desquamated renal tubular cells in the urine is used as a hallmark of recent intravascular hemolysis. Since hemosiderinuria is not usually found until several days after the onset of the hemolytic episode, when hemoglobinemia and hemoglobinuria may have disappeared, its presence can be helpful in establishing the presence of hemolysis retrospectively.[1]

Two procedures are currently used to demonstrate hemosiderinuria: a wet procedure, in which a drop of either unstained or Prussian blue–stained urine sediment is placed on a slide under a cover slip and examined microscopically; and a dry procedure, in which a push smear of the urine sediment is fixed and subsequently stained with Prussian blue prior to microscopic examination.[2] The unstained granules in a wet preparation may be difficult to detect, however, and the preparation is not permanent. The fixed smears of urine sediments often result in poor cellular morphology, which makes the intracellular iron difficult to find. In addition, the differentiation between extracellular iron granules and artifact is frequently challenging.[3]

The authors have developed a technique for preparing urine sediment smears using a cytocentrifuge (Cytospin 2)[a] that maintains excellent cellular morphology and facilitates the ready detection of intracellular hemosiderin.

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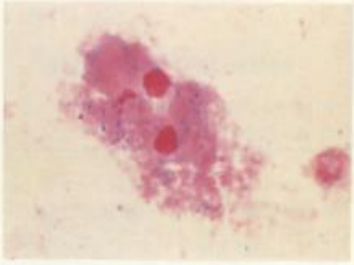
A 10-mL aliquot of fresh urine is centrifuged in a conical tube for five to ten minutes at 2,000 rpm. The supernatant is decanted, and the pellet is resuspended. Slides are prepared in duplicate. For each slide, 1 or 2 drops of the well-mixed sediment is added to a cytocentrifuge chamber, along with 1 drop of 5% albumin. When the urine specimen is very cloudy, 1 or 2 drops of unspun urine is used instead to avoid overly thick smears. The following routine cytocentrifugation procedure is used: the chambers are centrifuged for ten minutes at 1,250 rpm, after which the slides are removed, air dried, and fixed with 100% methanol for ten minutes.[4]

It is convenient to stain the slides with Prussian blue at the same time as the bone marrow specimens. For this procedure, equal volumes of 4% potassium ferrocyanide and 4% hydrochloric acid are mixed in a Coplin staining jar. The slides are immersed in this solution. An unstained slide of bone marrow from a specimen previously shown to have adequate iron is included as a control. After 30 minutes, the slides are removed and rinsed thoroughly with water. They are next counterstained for five minutes with a 0.03% basic fuchsin solution. Slides are then rinsed with water, rinsed briefly with absolute ethyl alcohol, and finally rinsed with water again.[3] After air drying, a drop of mounting media and a coverslip are placed over the reading area of each slide. Each slide is thoroughly examined microscopically using ×50 and ×100 oil lenses. Hemosiderin granules are easily recognized as blue granules in intact renal tubular epithelial cells and may also be seen extracellularly (Figure). Results are reported as positive or negative.



Positive urine hemosiderin. Distinct blue granules are apparent within the renal tubular epithelial cells.

This technique offers numerous advantages. First, the procedure is straightforward, and results in slides of uniformly good quality. In addition, the viewing area on the slide is small and readily located. Cellular morphology is well maintained and highlighted by the fuchsin counterstain. Finally, the fixed slides are permanent and may be stored for later review.

References

1. Pots I.D, Garratty G: *Acquired Immune Hemolytic Anemias*. New York, Churchill Livingston, 1980, pp 1–25.
2. Bradley M, Schumann GB: Examination of urine, in *Clinical Diagnosis and Management by Laboratory Methods*, ed 17. New York, W.B. Saunders Co, 1984, pp 380–458.
3. Beutler E: Peripheral blood, bone marrow, and urine iron stains, in Williams WJ, Beutler E, Erslev A, et al (eds): *Hematology*, ed 3. New York, McGraw-Hill Book Co, 1983, pp 1603–1604.
4. Shandon Cytospin 2 operating instructions. Sewickley, Pa: Shandon Southern Instruments, 1982.

Supplier

a. Shandon Southern Instruments, Sewickley, PA 15143.

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Document Control

Location of Master: Hematology Procedure Manual
Master electronic file stored on the Beaumont Laboratory server:
S:\HEMACOAG\Document Control\Hematology\Procedure\Master Documents\Urine Hemosiderin.doc
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: Nancy Ramirez, MT(ASCP)SH	02/1990			
Approved by: Joan C. Mattson, MD	03/1990			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C. Mattson, MD	12/18/1991		No change	
Joan C. Mattson, MD	12/28 /1992		OK	
Joan C. Mattson, MD	12/27/1993		Picture added; no change in protocol	
Joan C. Mattson, MD	12/12/1994		OK	
Joan C. Mattson, MD	12/20/1995		No change	
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Noelle Procopio, MT(ASCP)SH	12/05/2001		No change	
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Noelle Procopio, MT(ASCP)SH	12/22/2003		No change	
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Joan C. Mattson, MD	02/19/2005	00	Standardized procedure format	
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Ann Marie Blenc, MD	04/30/2007		No change; new director	
Ann Marie Blenc, MD	09/06/2007	01	Updated counterstain, pg. 1	
Ann Marie Blenc, MD	06/20/2008		No change	
Ann Marie Blenc, MD	03/12/2009		No change	
Ann Marie Blenc, MD	03/04/2010		No change	
Ann Marie Blenc, MD	05/03/2011		Procedure numbering only.	NA
Ann Marie Blenc, MD	05/23/2013	02	Added Attachments section to body of procedure.	NA
Ann Marie Blenc, MD	04/13/2015	03	Reformatted; updated Nuclear Fast Red counterstain	NA

Ann Marie Blenc, MD	10/19/2015	**04**	Updated procedure step to reflect Nuclear Fast Red Solution counterstain.	NA
Ann Marie Blenc, MD	04/14/2017		Logo update only.	NA
Elizabeth Sykes, MD	02/02/2018			
Ann Marie Blenc, MD	03/09/2018	**05**	Updated 10% HCL stability. Updated quality control section. Changed the verbiage of fixing the smears.	NA