

Urinalysis and Body Fluids 09

Unit 3

Chemical Examination of Urine

Part 6, Blood, Leukocytes, Nitrite & miscellaneous

Session Outline

- Blood
 - Hemoglobin
 - RBCs
 - Myoglobin
- Nitrite
- Leukocyte esterase
- Specific gravity
- Miscellaneous

Urine Blood

- **Hematuria** - Intact red cells in urine - *trauma / irritation of renal organs*
 - Glomerulus
 - Renal tubules
 - Ureters
 - Bladder
- **Hemoglobinuria** - Free hemoglobin in the urine
 - Glomerular filtrate
- **Myoglobinuria** - myoglobin in the urine
 - Glomerular filtrate

Urine blood - hematuria

- **Hematuria** - Intact red cells in urine
 - Possible Causes
 - Collagen diseases
 - Subacute bacterial endocarditis
 - Drugs - *toxic chemicals*
 - Hypertension
 - Urinary tract disorders - *kidney stones, glomerular nephritis, pyelonephritis*
 - Appendicitis
 - Anticoagulant therapy
 - Malaria
 - Hemoglobin from within cells reacts on dipstick
 - Microscopic positive for RBCs

Urine blood - hemoglobinuria

- **Hemoglobinuria** - Free hemoglobin in urine
 - Possible Causes
 - Burns, Crushing injuries, Toxins, incompatible blood transfusion,
 - Congenital and acquired anemias, Etc.
 - Pathophysiology
 - Free hemoglobin is picked up by **haptoglobin** to form a larger molecule that doesn't filter.
 - If the amount of hemoglobin exceeds the haptoglobin's capacity, free hemoglobin appears in the urine.
 - Appears when such extensive and rapid destruction of RBCs occurs, that the reticuloendothelial system cannot metabolize or store the free hemoglobin
 - Free hemoglobin is excreted in urine when serum levels exceed 100 mg per dl - threshold level
 - OR May be due to RBC lysis in urine itself

Chemical Exam of Urine - Blood

- **Hemosiderin**
 - A yellow-brown granular pigment derived from hemoglobin iron, deposits in the tissues.
 - Hemoglobin not picked up filters easily through the glomerulus
 - Some of it will be reabsorbed by the tubules, causing the formation of granules (hemosiderin) in the renal tubule epithelial cells
 - *Dr may ask for iron stain on urine sediment*
 - Granules appear yellow-brown and may be free in the urine as well as inside the epithelial cells
 - To demonstrate hemosiderin, perform a Prussian-blue reaction / stain

Urine - myoglobinuria

- **Myoglobin** - the heme portion of striated muscle.
- Most well known, non-hemoglobin substance that reacts on blood dipstick.
 - Ferrous porphyrin similar to hemoglobin
 - Lower molecular weight
 - Only one iron molecule
 - Not normally present in plasma
 - Damage to cardiac or skeletal muscle
 - Crush injuries
 - Electrical shock
 - Myocardial infarction
 - Effects of toxins
 - others

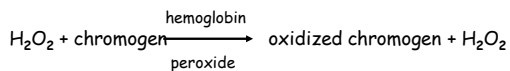
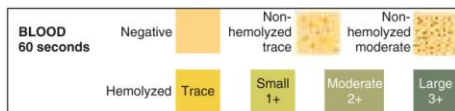
Urine blood/hemoglobin vs myoglobin



- Both, hemoglobin and myoglobin will produce positive reagent strip blood results
- Macroscopic differentiation
 - Red plasma plus red urine equals hemoglobin
 - Normal looking plasma plus red urine equals myoglobin
- Chemical differentiation
 - Ammonium sulfate test
- Protein electrophoretic mobility - most definitive

Urine blood/hemoglobin

Blood color chart



Chemical Exam of Urine - Blood

- Sensitivity
 - can detect a small number @ # 5-10 RBC/ uL
- Interfering substances
 - False positives
 - Menstrual blood
 - Bacterial peroxidases
 - Strong oxidizing agents such as bleach
 - False negatives
 - *Ascorbic acid - noted to suppress reaction
 - High concentration of nitrates
 - Specimens with high specific gravity (cells crenated)
 - Specimen not mixed well

Urine blood/hemoglobin: significance

- Significance
 - Hematuria
 - Hemoglobinuria
 - Myoglobinuria
- Always correlate results with microscopic.

Chemical Exam of Urine - Nitrite

- Principle / *purpose of testing*
- Certain species of bacteria convert nitrate (normal constituent of urine) to nitrite
 - Escherichia - most common cause of UTI
 - Klebsiella
 - Proteus
 - Pseudomonas
 - Enterobacter
 - Citrobacter
- Under certain conditions, the presence of urinary nitrite may indicate urinary tract infection
- *Test used to detect possible urinary tract infections, & to evaluate the effectiveness of subsequent antibiotic therapy.*
- *Screening test only, not to replace microbiology cultures*
- *On negative specimens, if clinical symptoms are present, physician should proceed with cultures.*

Chemical Exam of Urine - Nitrite

- Aromatic amine in reagent strip reacts with nitrite; producing a diazonium salt
- The diazonium salt reacts with sulfanilic acid and acetic acid to produce a pink azo dye
- Sensitivity
 - N-Multistix 0.075 mg/dl nitrate ion
 - Chemstrip 0.050 mg/dl nitrate ion

Nitrite 90 seconds Negative Positive (Any degree of uniform pink colour is positive)

Chemical Exam of Urine - Nitrite

- Limitations
- Qualitative results
 - Reported as positive or negative
 - Sensitivity standardized to correspond to @ 100,000 organisms per mL.
- Negative result does not rule out infection
 - Fresh, first morning specimen very important.
 - UTIs caused by organisms that do not convert nitrates to nitrites not detected.
 - Haemophilus
 - Staphylococcus
 - Streptococcus

Chemical Exam of Urine - Nitrite

- False negatives
 - Pathogen non-nitrate reducer
 - Urine in bladder insufficient time
 - Urine that has a very high specific gravity / urobilinogen / ascorbic acid.
 - No nitrate in the diet
 - Nitrite was further reduced to nitrogen gas
- False positives
 - Old urine specimens - *contaminating bacteria will grow in specimen, and in the process convert...*
 - Medications that color the urine

Chemical Exam of Urine - Leukocyte esterase

- **Leukocyte Esterase**
- Leukocyte esterase testing is another test used as a means of screening for urinary tract infection.
 - Screening for UTI involves odor, pH, protein, leukocyte esterase and nitrite.
- Does not measure concentration of leukocytes
- Will detect presence of lysed leukocytes as well as intact WBCs

Chemical Exam of Urine - Leukocyte esterase

- **Test Principle**
 - Leukocyte esterase, an enzyme present in granulocytes, hydrolyzes indoxylcarbonic acid esterase to produce indoxyl, which reacts with a diazonium salt to create a purple color usually in 2 min.
- **Reaction interference**
 - False positives - oxidizing detergents
 - False negatives - greatly increased glucose, protein, or specific gravity.
 - Increased specific gravity could cause WBC to crenate preventing their releasing their esterase. So it is possible for the dipstick to be negative when there are WBCs present.
 - Inaccurate timing - must allow the 2 minutest before quantitating.
 - Specimens must be at room temp and well mixed.



Chemical Exam of Urine - Specific gravity

- Measures pKa change of polyelectrolytes in relation to ionic concentration; actually measures ionic concentration
- Relates to urine specific gravity.
- When more ions are present, more acid groups become disassociated, releasing hydrogen ions and causing the pH to change.
 - Indicator (bromthymol blue) then measures the change in pH.
- When urine has an increased specific gravity, the reagent pad becomes more acidic.