

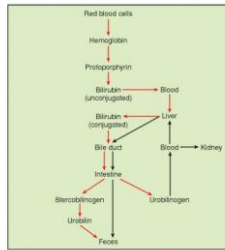
Urinalysis and Body Fluids CR9

Unit 3
Chemical Examination of Urine

Part 5, Bilirubin and Urobilinogen

Chemical Exam of Urine - bilirubin

- Bilirubin and Urobilinogen - presence in urine may be the 1st indication of liver disease
- Bilirubin formation (overview)
 - Formed by hemoglobin degradation
 - RBCs breakdown @ 120 days
 - Iron, proteins & amino acids are recycled
 - Protoporphyrin (heme) eventually becomes bilirubin.
 - Liver's reticuloendothelial cells (Kupffer) & liver parenchymal cells (hepatic cells)



Chemical Exam of Urine - bilirubin

- Free bilirubin (insoluble, indirect, unconjugate)
 - Formed first
 - Circulates in blood bound to protein - *albumin* - which adds to its size - keeping it from being excreted
 - ****Water insoluble** - cannot be excreted by kidney

Chemical Exam of Urine

- Insoluble bilirubin
- Goes to liver - converted to water soluble bilirubin by the liver parenchymal (hepatocytes) cells
 - conjugated with glucuronic acid
 - Forms bilirubin diglucuronide (*also called direct bilirubin, conjugated bilirubin, or water soluble bilirubin*)

Chemical Exam of Urine

- **Conjugated or direct bilirubin** - also called bilirubin diglucuronide or direct bilirubin
- Most excreted through bile duct into the intestine
- Small amounts of conjugated bilirubin regurgitate back from the bile duct and into the blood system (not more than 0.2 - 0.4 mg/dL).
- Get filtered through the glomerulus and excreted in the urine whenever the plasma level is increased.
- Normally, no detectable amounts are present in urine

- Majority goes down bile duct - into bile - into intestine

Chemical Exam of Urine

- Urobilinogen
- Formed in intestine as a result of the bacterial action on the conjugated / direct bilirubin
 - The conjugated bilirubin is degraded to form stercobilinogen and urobilinogen.
 - The urobilinogen formed is absorbed by intestine and returned to liver by portal circulation
 - - *where it is filtered out to again be pushed down into the intestines.*
 - Most of which will be returned to intestine again
 - A small amount, @ 1%, escapes the liver clearance and will be excreted into urine.
 - Urobilinogen / stercobilinogen in the intestine / bowel will be reduced again (by bacteria) to form urobilin.
 - *Bacteria continue to act on it to reducing it - forming urobilin - feces normal brown color*

Chemical Exam of Urine

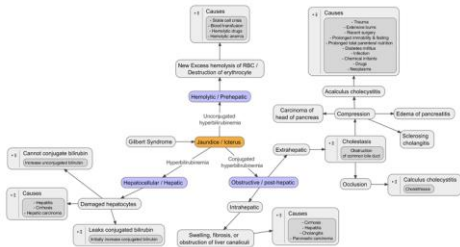
- **Jaundice**
- Condition when serum bilirubin becomes greater than the liver can handle, and there is an abnormal collection of bilirubin in the tissues giving them a yellow color - *eyes, skin, urine, serum etc. has a very distinctive yellow color.*



Wikipedia

Jaundice - types

Wikipedia



Jaundice

- **Hemolytic jaundice**
- Excessive hemolysis of red cells (sickle cell anemia, cold agglutinins, malaria, burns, mechanical heart valves, etc.)
 - Liver functions normal - conjugates and eliminates bilirubin
 - Too much bilirubin produced - liver can't clear blood *resulting in a build up of indirect, insoluble bilirubin*
- No bilirubin found in urine - *why?*
- Increased urobilinogen found in urine
 - the liver is occupied with conjugating indirect bilirubin
 - can't reprocess the urobilinogen that is coming from the intestines.
 - So the urobilinogen goes into the urine

Jaundice

- **Hemolytic jaundice clinical picture**
- Negative urine bilirubin
 - - *the serum level of unconjugated bilirubin is increased, but it is not soluble*
- Increased urine urobilinogen
- Increased fecal urobilinogen

Jaundice

- **Obstructive jaundice**
- Causes *some type of blockage* (Gall stones, Tumor, Edema)
- Liver conjugates but can't excrete
- Conjugated bilirubin regurgitated into blood
- Conjugated (direct) bilirubin found in urine
- No urobilinogen found in urine
- No urobilin in feces

Jaundice

- **Obstructive jaundice clinical picture**
- Positive urine bilirubin
- Negative urine urobilinogen
- Negative-trace fecal urobilinogen
- *stools are a grayish, chalky, clay color.*

Jaundice

- **Hepatocellular jaundice**
 - Malfunction of liver cells *ie. viral hepatitis, cirrhosis, etc.*
 - Both urobilinogen and bilirubin (*direct*) found in urine
- **Hepatocellular jaundice clinical picture**
 - Positive urine bilirubin
 - Normal fecal urobilinogen
 - Increased urine urobilinogen

Chemical Exam of Urine

- Testing for Bilirubin
- **Review questions:**
 - If specifically interested in detecting urine bilirubin / urobilinogen, what special collection and handling requirements are necessary? & Why?
 - What physical characteristics would you expect a sample containing bilirubin to have?
 - What about its microscopic sediment?

Chemical Exam of Urine - bilirubin

- If specifically interested in detecting urine bilirubin / urobilinogen what special collection and handling requirements are necessary? & Why?
 - Because bilirubin is easily destroyed in light and air, great care should be taken to protect it from these factors. The specimen should also be transported and tested ASAP. Testing specific for urobilinogen requires collection from 1-3 PM.
- What physical characteristics would you expect a sample containing bilirubin to have?
 - Dark, amber color. Yellow foam.
- What about its microscopic sediment?
 - Bilirubin stained sediment, bilirubin crystals, others?

Testing for bilirubin

Most current tests for bilirubin are based on a diazo reaction.

- **Ictotest** - bilirubin confirmatory test
- Reagents
 - Diazo
 - Sulfanilic acid - provides suitable acid environment
 - Naphthylamines
- Procedure overview:
 - Add drops of urine to a special asbestos mat
 - Bilirubin, if present in the urine, remains on outer edge of mat.
 - Place a tablet on top,
 - Add drops of water to the tablet,
 - Allow it to spill over onto the mat,
 - Look for a purple color development on the mat.

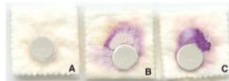


Urine bilirubin confirmatory test

Most current tests for bilirubin are based on a diazo reaction.

Ictotest

- Reagents
 - Diazo
 - Sulfanilic acid - provides suitable acid environment
 - Naphthylamines
- More sensitive
 - As little as 0.05mg/dL
- More specific
 - Less interference
 - Generally easier to read

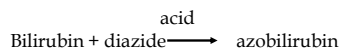


Ictotest color reactions.

Urine dipstick bilirubin

Bilirubin color chart

BILIRUBIN 30 seconds	NEG	Small 1+	Moderate 2+	Large 3+
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Sensitivity = 0.5 mg/dL

Urine bilirubin: false positive

- Technique errors
 - Reading after the prescribed time
- atypical color reactions produced by:
 - Indican
 - Metabolites of etodolac (Lodine)
 - Chlorpromazine (Thorazine)
 - Metabolites of phenazopyridine
- Confirm results with Ictotest

Urine bilirubin: false negative

- Large amounts of ascorbic acid decrease the sensitivity
- High levels of nitrite
- ***Exposure to light and room temperature
 - Bilirubin oxidizes to biliverdin

Urine urobilinogen formation

- May indicate **liver disease** or **hemolytic disorders**
- Review of formation
 - Formed in intestine from conjugated bilirubin
 - Bacterial enzymes convert bilirubin to urobilinogen (colorless) then further reduce it to urobilin (brown)
 - 10 - 15 - 50% (authors vary) gets reabsorbed into the blood where most of it is recaptured by the liver and returned to intestines.
 - Small amount escapes liver filtration and filters through glomerulus to be found in the urine.

Urine urobilinogen: significance

- Normally present in low amounts
 - 1-4 mg/24 hr (< 1.0 Ehrlich unit/ 2 hr)
- Peak levels between 2-4 pm
 - When specifically testing for urobilinogen,
 - must collect 2 hr urine (timed sample) from 1-3 or 2-4 pm
 - Specimen must be tested immediately
- Elevated in
 - Liver disease
 - Intestinal obstruction
 - Hemolytic anemia
 - Hemolysis

Urine urobilinogen

Urobilinogen color chart

UROBILINOGEN	Normal	0.2	1	Abnormal	2	4	8
60 seconds		mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL

****p*-Dimethylaminobenzaldehyde + urobilinogen = azo dye
(Ehrlich's reagent)

Urine urobilinogen: false positive

- *p*-aminosalicylic acid
- sulfonamides,
- *p*-aminobenzoic
- Prophobilionogen
 - May use Watson-Schwartz test to differentiate
- ** any dye or substance that will stain the dipstick pad
 - Phenazopyridine (Azo-Gesic, pyridium etc.)

Urine urobilinogen: false decrease

- A true absence of urobilinogen is not detectable.
- Loss of intestinal bacteria
 - The conjugated bilirubin would not be converted to urobilinogen
 - patient taking broadspectrum antibiotics
- Biliary obstruction
 - No bilirubin getting into intestines
- Improper storage of specimen
 - oxidation of urobilinogen to urobilin
- Urine Nitrites and formalin preservatives

Urobilinogen testing

- Porphyrins - chemical intermediates in the synthesis of cytochromes (such as hemoglobin, myoglobin, etc.)
- Porphyrrias - inherited or acquired enzyme deficiency that result in overproduction of a heme precursor resulting in build up in the blood, feces and urine
 - ***Lead poisoning most common cause of acquired porphyria
- Porphobilinogen - not normally found in urine. **When present causes the urine to have "port red wine" color (red diapers)
 - Related to urobilinogen - and reacts with Ehrlich's reagent
- Tests to differentiate urobilinogen from porphobilinogen
 - Watson- Schwartz Differential test
 - Urobilinogen extractable into chloroform and butanol
 - Hoesch Screening test
 - Does not react with urobilinogen

Reference Listing

- Please credit those whose work and pictures I have used throughout these presentations.
- Lillian Mundt & Kristy Shanahan, Graff's Textbook of Urinalysis and Body Fluids, 2nd Ed.
- Susan Strassinger & Marjorie Di Lorenzo, Urinalysis and Body Fluids, 5th Ed.
- Wikipedia, the free encyclopedia
 - www.wikipedia.org
