URINE DIPSTICK AND SULPHOSALICYLIC ACID TEST

Špela Borštnar
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KIDNEY DISEASE?

- severity of kidney disease = estimating GFR
- cause of kidney disease = urinalysis
1. macroscopic urine examination
2. dipstick test and sulphosalicylic acid test
3. quantitative and qualitative measurement of proteinuria
4. urine sediment examination - urine microscopy
DIPSTICK TESTING

- reagent strip – dipstick
- rapid semi quantitative assessment of urinary characteristics
- manual method
- method with automatic machines
- different dipsticks – be aware
DIPSTICK TESTING

- specific gravity
- pH
- leukocytes
- nitrite (bacteria)
- protein (albumin)
- glucose
- ketones
- pigments (bilirubin, urobilinogen)
- hemoglobin (erythrocytes)
SPECIFIC GRAVITY

- defined as the weight of the solution compared with the weight of an equal volume of distilled water
- gives information about concentration ability of the kidney

- dipstick detects only ionized substances and not glucose or X ray contrast agent → measured specific gravity of urine is lower
- when is low (1.005) → cells can burst → the dipstick can detect cells, but under the microscope they can not be seen

- we can measure: 1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030
pH

- reflects the degree of acidification of the urine
- pH: 4.5 – 8.0, average 5.0 - 6.0 – slightly acidic
- we can measure: 5, 6, 7, 8, 9

**Why is important to know pH of the urine?**
- in metabolic disorders (mtb acidosis)
- in renal disorders
- in patient with kidney stones
- in patient with urinary tract infection
LEUKOCYTES - LEUKOCYTE ESTERASE

- presence of neutrophils and macrophages is indicated by a positive leukocyte esterase test

- we can measure: neg, 1+, 2+, 3+
false-positive results:
- strong oxidizing agents (strong detergents)
- vaginal discharge
- some preservatives (formalin)
- drugs: nitrofurantoin, imipenem, meropenem, clavulanic acid
- excessively dilute urine
LEUKOCYTES - LEUKOCYTE ESTERASE

- false-negative results
  - concentrated urine
  - proteinuria
  - glycosuria
  - drugs (ascorbic acid, oxalic acid, gentamicin, tetracycline)
- **Nitrite test** is a rapid, indirect method for detection of significant and asymptomatic *bacteriuria*.

- Some bacteria: Enterobacter, Citrobacter, E. Coli, Klebsiella, and Proteus reduce *nitrate to nitrite*.

- Analyze first morning urine.

- We can detect: positive (any degree of uniform pink color) or negative.
NITRITE

- false-positive results
  - urine stands too long before testing
  - red urine
false-negative results

A negative test should never be interpreted as indicating the absence of bacterial infection!!!

Why?

- presence of pathogens in the urine that do not form nitrite
- the urine was not in the bladder long enough
- the urine does not contain any nitrate
- the bacterial enzymes may have reduced nitrate to nitrite and then converted nitrite to nitrogen

- urobilinogen, ascorbic acid, acidic urine
GLUCOSE

- the presence of glucose in the urine – *glycosuria – glucosuria*

- **causes:**
  - inability of kidney to reabsorb filtered glucose despite normal plasma glucose levels – *renal glycosuria*
  - urinary spillage because of abnormally high plasma glucose concentration

- **two types of test:**
  - with the enzyme glucose oxidase - specific for glucose
  - copper reduction test – detects any reducing substance

- we can detect: negative, 1+, 2+, 3+, 4+ - be aware with interpretation !!
- **false-positive results**: not likely
  - a reading taken after the prescribed time
  - urine contamination with strong oxidizing cleaning agents
- **false-negative results**
  - sensitivity for glucose may be affected by temperature, specific gravity and pH
  - *ascorbic acid*: ingestion, parenteral administration of vitamin C or antibiotics that contain ascorbic acid as a stabilizing agent (tetracycline)
  - moderately high ketone levels may reduce sensitivity
KETONES

- **ketones or ketone bodies** – formed during the catabolism of fatty acids
- acetoacetic acid, β-hydroxybutyric acid and acetone
- some reagents strips are sensitive only to acetoacetic acid, some also detect acetone, none detect β-hydroxybutyric acid
- normal no ketones in the urine, except in starvation, extreme exercise, diabetic ketoacidosis

- we can detect: neg, 1+, 2+, 3+
KETONES

- **false-positive results**
  - highly pigmented urine specimen
  - large amounts of levodopa metabolites
  - specimens with high specific gravity and a low pH

- **false-negative results**
  - delayed analysis of urine
- **bilirubin**: no detectable amount of bilirubin in urine

- **high levels of bilirubin in plasma**:  
  - ↑ unconjugated bilirubin → no bilirubin in the urine  
  - ↑ conjugated bilirubin → bilirubin in the urine (*early detection!*)

- **bilirubin** - light sensitive – protect the urine from the light, quickly examination

- **bilirubin** - yellow brown → **biliverdin** – green
we can detect: neg, 1+, 2+, 3+

- **false-positive results:**
  - chlorpromazine, metabolites of some drugs

- **false-negative results:**
  - ascorbic acid
  - high levels of nitrite
  - exposure to room temperature and light
PIGMENTS - UROBILINOGEN

- **urobilinogen**: a very small amount of urobilinogen is excreted by the kidney into the urine

- **altered levels of urobilinogen in urine:**
  - liver disease
  - antibiotics (alter normal bacterial flora)
  - intestinal obstruction

- **instability**: urobilinogen → urobilin
PIGMENTS - UROBILINOGEN

- most dipsticks show block(s) for normal levels and blocks for elevated levels
- we can detect: norm, 1+, 2+, 3+, 4+

- **false-positive results:**
  - substances that react with urobilinogen test pad (sulfonamides,..)
  - urine from patients receiving phenzopyridine

- **false-negative results:**
  - urine containing nitrite or those preserved with formalin
  - improperly stored samples
HEMOGLOBIN - ERYTHROCYTES

- heme – acts like pseudoperoxidase and reacts with peroxide and a chromogen on the test pad
- positive results in hematuria, presence of free hemoglobin (intravascular hemolysis - hemoglobinemia) or myoglobin
- the diagnosis of hematuria → conformation with microscopy (be aware in high pH and low SG)

- two separate color scales: for erythrocytes and hemoglobin
- we can detect: ery: neg, 1+, 2+, 3+, 4+
  hb: 1+, 2+, 3+, 4+.
- **false-positive results:**
  - oxidizing contaminants (hypochlorites)
  - high bacterial content
  - semen in the urine
  - menstrual blood
false-negative results: unusual → negative dipstick for heme theoretically excludes hematuria

- the urine is not mixed well
- ascorbic acid
PROTEINURIA < 150 mg/day

Bowman's space

glomerular basement membrane

HMW proteins
albumin
LMW proteins

tubular cells
PROTEINS

- white foam – indicator of proteinuria
PROTEINS

- urine dipstick test is sensitive to albumin – semi quantitative means of assessing albuminuria
- albuminuria in the range of 30 – 300 mg/L in most cases cannot be detected
- detection of albuminuria more than 300 mg/L
- positive dipstick → proteinuria should be quantified
- dipstick is insensitive to non-albumin proteins (be aware of immunoglobulin light chains)

- we can detect: 0, 1+, 2+, 3+, 4+
- **false-positive results:**
  - very concentrated urine
  - highly alkaline urine
  - ammonium compounds (for cleaning urine containers)
  - contamination with vaginal discharge, semen, heavy mucus, pus and blood
  - chlorhexidine gluconate, phenazopyridine,..
PROTEINS

- false-negative results:
  - very dilute urine
  - proteins other than albumin!!!
SULFOSALICYLIC ACID (SSA) TEST

- SSA detects all proteins in urine (including light chains and albumin)
- the test – mixing urine supernatant with SSA
- if becomes turbid, this indicate the presence of proteinuria
SULFOSALICYLIC ACID (SSA) TEST

0 = no turbidity (0 mg/dL)
trace = slight turbidity (1 to 10 mg/dL)
1+ = turbidity through which print can be read (15 to 30 mg/dL)
2+ = white cloud without precipitate - heavy black lines can be seen (40-100 mg/dL)
3+ = white cloud with fine precipitate - heavy black lines cannot be seen (150-300 mg/dL)
4+ = flocculent precipitate – cloud is dense with large clumps (more than 500 mg/dL)
SULFOSALICYLIC ACID (SSA) TEST

- **false-positive results**
  - therapy with tolbutamid, penicillin, cephalosporine, sulphonamide
  - investigation with iodine radiocontrast agent (up to three days)

- **false-negative results**
  - highly alkaline urine
  - very diluted sample
Dipstick testing + precipitation test

- Dipstick testing ≥1 and precipitation test positive → U-proteins/U-creatinine

- Dipstick testing 0 and precipitation test positive → S-light chains? tubular proteinuria?

- Dipstick testing 0 and precipitation test 0
WORKSHOP tomorrow

- URINE DIPSTICK, SULPHOSALICYLIC ACID AND SEDIMENT PREPARATION – HANDS-ON SESSION