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Analysis of urine and urinary sediment

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What will it be about?

1. Basic urine examination
2. Physical examination of urine (volume, color, odor, turbidity, foam, density, osmolality)
3. Chemical examination of urine
   a) pH
   b) nitrites
   c) leukocytes
   d) protein(s)
   e) glucose
   f) ketone bodies
   g) blood (erythrocytes, hemoglobin, myoglobin)
   h) bile pigments (bilirubin, urobilinogen)
4. Urine sediment analysis (morphological urine examination)
   a) Cellular elements
   b) Cylinders
   c) Microorganisms
   d) Crystals

1. Basic urine examination

Urine is

- readily available biological fluid
- analysis → obtaining information about the state of the organism, its metabolism, and kidney function
- Formation: Blood → glomerulus → filtration through the basement membrane → ultrafiltrate (primary urine) is drained through the tubules → bladder. In the tubules →some components are resorbed (glucose, Na, Ca, phosphates, hydrogen carbonate, AMK, etc.) → some secreted (potassium, protons, organic anions, and cations, at higher concentrations creatinine, etc.) → and thickening of urine by water resorption. The result is → definitive urine.
- We perform a basic urine examination:
  - As targeted - when a certain disease is suspected (with pathological urinary manifestations)
  - non-targeted (screening in asymptomatic persons)
  - a routine part of panels of entrance examinations (before hospitalization).
- Basic urine examination is divided into:
  - **physical**
  - **chemical**
  - and **morphological examination** (urine sediment)

1. Basic examination of urine - sampling

- **Correct urine collection procedure:**
  - Genital cleansing
  - Medium stream of morning urine
  - Chemical examination within 2 hours, sediment within 1 hour
  - Urine collection - beginning, end, preservation
- The most informative values collected from the first-morning urine sample are the most telling.
- Time-based urine collection:
  - For quantitative analyzes and clearance determination of various analytes
  - Time interval collection: short-term urine collection takes 1-3 hours, long-term 12-24 hours.
  - After completion→ the volume of urine is measured, the urine is thoroughly mixed, and a minimum of 5 ml of an average sample is delivered to the laboratory, with information on the exact start and end time of collection (accurate to the minute) and with information on the exact volume of collected urine.
  - Urine collected over a longer period of time (e.g. 24 h) must be preserved. Today, urine collection is often abandoned due to the unreliability of the method.

2. Physical examination of urine

- Physical examination refers to the sensory evaluation **of the color** , **turbidity**, **foam,** and **odor** of urine.
- **Density**, **volume** and **osmolality** are determined instrumentally (or with an indicator paper) .

2a) Color

- Different colors are caused by excreted bile pigments and their metabolites.
- The color of urine is also affected by some ingested vitamins, drugs, and other xenobiotics.

Colour (Examples)	Cause
Light to colorless	Polyuria in excessive drinking, diabetes, the renal regulatory function failure
Yellowish-brown, brownish or rusty	Lack of water, water loss
Pure Yellow	Flavins, riboflavin (B_2), vitamin mixtures
Red	Porphyrins, haemoglobin, myoglobin, blood, organic pigments, plant pigments (turn blue in alkaline environment)
Brown-red	Urobilinogen (often with bilirubin)
Brown	Bilirubin, hematin, methemoglobin, melanin
Green, blue	Organic compounds

The colour of urine is based on urinary pigments – yellow urochrome and red urorosein; different shades depending on the amount of water; the structure of urorosein is e.g.
http://www.pubmedcentral.nih.gov/pagerender.fcgi?artid=1197863&pageindex=1#page

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2. Physical examination of urine

2b. Urine odor:

- old urine, diseases accompanied by stagnation of urine → ammonia smell
- in case of ketoacidosis → acetone is smelled
- with proteinuria and hematuria → urine smells putrid (caused by the release of hydrogen sulfide from amino acids containing –SH groups)
- in hereditary mtb disease leucinosis → it smells like maple syrup.

Odor

It is detected in fresh urine after shaking; Normally, an aromatic smell of beef soup is characteristic.

Cause	Odor
Food (alimentary)	Garlic, fruit, alcohol
Metabolic disorders	Acetone (diabetes mellitus, starvation)
Exogenous substances (toxic)	Organic solvents, etc.
Urinary tract infections	Ammonia (urease bacteria), putrefactive bacteria
In old urine, the cause of the smell is decay, putrefaction, fermentation	

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2. Physical examination of urine: foam, turbidity

- Sensory evaluation, with the exception of color and turbidity evaluation, is mostly abandoned today. Unusual colouring, turbidity → noted in the report.

Foam

On the healthy person urine, the foam is white, unstable.

Foam	Cause
More abundant, colorless	Proteins, glucose, detergents (!)
Yellow to yellow-brown	Bilirubin
Bubbles in fresh urine	Urinary tract infections

Turbidity

Normal urine is clear, the physiologically present *Tamm-Horsfall* mucoprotein (so-called *nubecula*)) *falls out of the cooling urine*.

Turbidity	Sediment	Cause
White	Reddish	Urates, uric acid
White	White	Phosphates, ammonium ureate, carbonates, uric acid, oxalates [*distinction using HAc and HCl*]
Yellow	-	Certain amino acids (leucine, tyrosine)
Yellowish	Raged	Leukocytes, bacteria, yeasts
Smoky, reddish, rusty	Red to brown	Blood
White	-	Fat (exceptional find)

Source: https://postudium.cz/Analaza_moci_CEVA_2013.pdf

2. Physical examination of urine: urine volume

- is significantly influenced by fluid and food intake. Volumes less than 400 ml/24 hours and more than 2500 ml/24 hours are considered pathological.
- **Oliguria**, **anuria**: Oliguria refers to the volume of urine **< 400 ml/24** hours and anuria to the amount of urine **< 100 ml/24 hours**.
  - The basic symptom of kidney failure, the cause may be:
    - dehydration (↓ fluid intake or increased loss (diarrhea, sweating);
    - primary renal parenchyma damage, the result of fluid retention (edema, effusions in body cavities); mechanical obstruction - urinary tract (prostate hypertrophy, wedged stone(s), tumors...)

Quantity

Standard: 600 – 2500 ml
Men: 1500 – 2000 ml
Women: 1200 – 1500 ml

- **Polyuria:** increase in daily diuresis above 2500 ml, 2 types:
  - caused by *the so-called water diuresis* → reduction of tubular water resorption X tubular resorption and excretion of osmotically active substances (OAL) is within normal limits. Physiologically → when taking in ↑ amount of water; with ↓ antidiuretin secretion (diabetes insipidus).
  - caused by *the so-called osmotic diuresis* → either increased OAL filtration (as a result of their increased osmotic concentration in ECF (e.g. hyperglycemia) or their reduced tubular resorption. Unabsorbed OAL → "bind" water → decrease in the tubular resorption. Typical for diabetes mellitus, polyuric phase of renal failure, and diuretic therapy.

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2. Physical examination of urine: urine density

- Relative density (also ***relative specific gravity***) is determined by the mass concentration of all solutes excreted in the urine. It is dependent on the number of dissolved particles and their molecular weight (unlike osmolality). Under physiological conditions, urine density (UD) ranges from **1.015–1.025**; in children between 1.006 - 1.014. Falsely higher values are caused by proteinuria above 1g/l alkaline urine pH.

- In a healthy individual, it is related to the urine volume: the ↑volume, the lower the UD (diluted urine) X with ↓volume increases (concentrated urine) → an indicative indicator of fluid intake
- The density of urine allows the estimation of the concentration capacity of the kidneys. Values above 1.020 and above are an indicator of good renal function. Highly concentrated urine indicates a substantial reduction in circulating plasma volume.
- Isosthenuria is a serious symptom of kidney damage. The kidneys lose the ability to concentrate (and dilute) urine and excrete urine with the same density as the density of the glomerular filtrate.

Changes in the relative urine density

Term	Relative density	Causes
Eustenuria	1,020–1,040	
Hyperstenuria	> 1,040	• dehydration • glycosuria • proteinuria
Hypostenuria	< 1,020	• diabetes insipidus • hyperhydration • renal failure • diuretics

Source: https://www.wikiskripta.eu/w/Vysetrenimoci

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2. Physical examination of urine: osmolality

- It depends on the amount of osmotically active particles excreted in the urine, while their weight, size and electrical charge do not matter.
- Osmolality is expressed in mmol/kg. It is only approximately dependent on the density of the urine. Its measurement is more accurate compared to density (↑ informative value, preferred).
- Comparison: osmolality reflects the total molar concentration of all solutes, X density their total mass concentration. I.e., a change in the concentration of low-molecular substances (especially Na, Glc , urea) will affect the osmolality more, while the presence of protein in the urine will have a more significant effect on density.
- Normal osmolality values with normal fluid intake are 300–900 mmol/kg. Urine osmolality depends on the dilution and concentration ability of the kidneys.
- Impaired renal concentration ability is one of the first signs of kidney disease (examination: measurement of osmolality in the morning urine sample and concentration (*adiuretin)* test – kidney response to the administration of antidiuretic hormone).

3. Chemical examination of urine

- Chemically, urine is examined mainly qualitatively (or semi-quantitatively), some analytes can also be determined quantitatively (protein, Glc-limited).
- Chemical examination can be performed within **4 hours after sampling** .
- **pH** , **nitrites, leukocytes, protein, glucose, ketone bodies, blood and bile pigment**
- Chemical examination of urine using liquid reactions in a test tube have been replaced by methods based on dry chemistry - test strips.
- Test strips: commercial monovalent or polyvalent **test strips** containing zones impregnated with reagents that provide a color reaction with the particular urine component.
- Evaluation: visually (by eye, subjective, the color is compared with the attached color scale) or objectively - instrumental evaluation (reflective photometry) - preferred, ↓error rate in the evaluation.
- Reflectance photometers - measure the intensity of light of selected wavelengths reflected from the relevant zone of the test strip. Some photometers are now automated (+ automated microscopic part) → a line for automated complex urine analysis, i.e. a urine analyzer.

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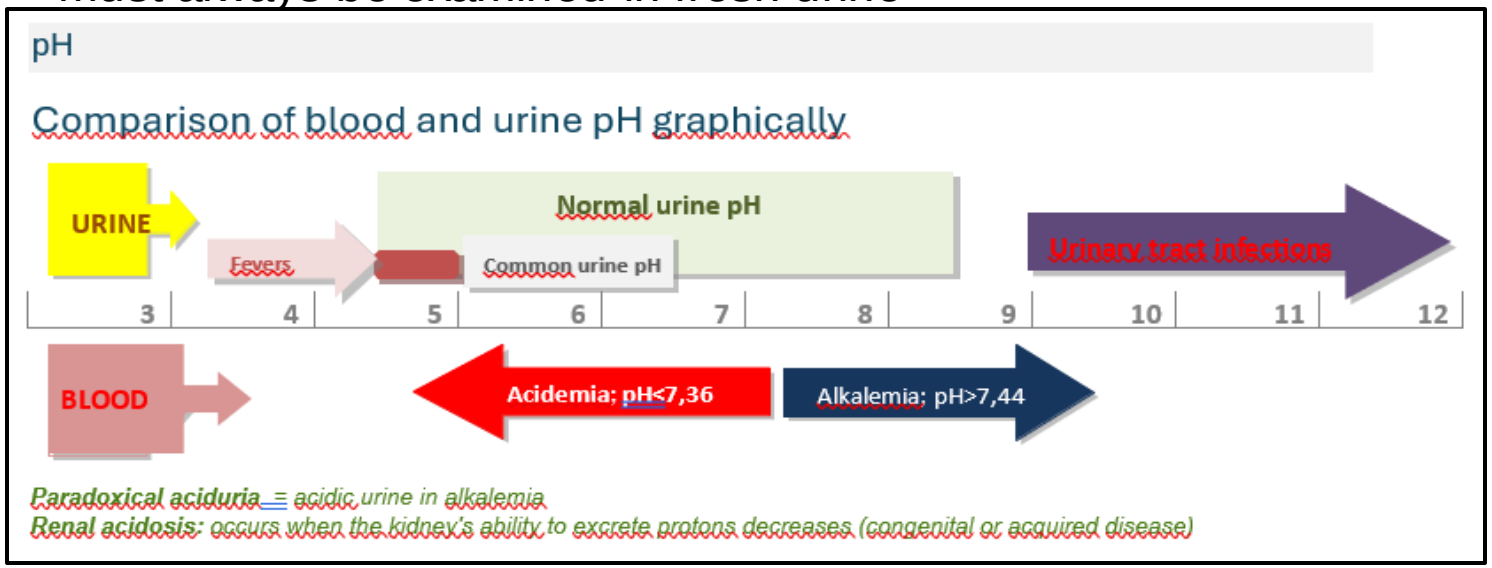
3. Scope of chemical urine examination

A typical test strip contains zones for determining:

- 1. pH
- 2. Density
- 3. Leukocytes
- 4. Nitrites
- 5. Proteins
- 6. Glucose
- 7. Ketones
- 8. Blood (erythrocytes)
- 9. Bilirubin
- 10. Urobilinogen

3a) Urine pH

- Kidneys → adjusting the acid-base balance of the organism by excretion (or retention) of H+. In glomerular filtrate, the pH is the same as in plasma. → When the filtrate passes through the renal tubular system→ acidification of urine.
- The concentration of free H+ in urine is insignificant → H+ is eliminated by the kidneys in two forms:
  - bound to the anions present (e.g. to phosphates: $H^+ + HPO_4^{2-} \rightarrow H_2PO_4^-$)
  - as ammonium cation ($H^+ + NH_3 \rightarrow NH_4^+$)
- The pH of the urine depends on - the diet composition
  - the state of acid-base balance
- must always be examined in fresh urine



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3a) Urine pH

The main benefit of urine pH testing is in the diagnosis and treatment **of urinary infection** and **urolithiasis**.

1. A consistently **alkaline urine pH** may indicate:

- Bacterial renal infection (urinary tract) → produce urease → hydrolysis of urea → ammonia is produced → alkalization of urine.
- Distal type of renal tubular acidosis = tubular cell disorder → inability of the distal tubule to excrete H+ (the acidification capacity of the kidneys is impaired).

2. Formation **of urinary stones** (urolithiasis):

- In acidic urine → usual stones: from calcium oxalate $Ca(COO)_{2;}$ from uric acid.

- Phosphates are poorly soluble in alkaline urine.

Normal blood pH	7,36 - 7,44	**Normal urine pH**	4,5 – 8,5 (7,5 podle Racka)
Acidemie:	pH < 7,36	**Common urine pH**	5,0 – 6,5
Alkalemie:	pH > 7,44	**Diet impact**	plant based- alcalization;animal based - acidification
		pH pod 4,5:	does not occur
		pH 4,5 – 5,0:	fevers
		Pathological pH	alcalic
		pH nad 9:	- renal or urinary tract infection - old urine secondary infections
		Onother causes of alcaline urine	inability of renal tubular cells to reabsorb bicarbonates (renal tubular acidosis)

Determination of pH

- approximate - using indicator papers/strips (colorimetry)
- accurate measurement - pH-meters (potentiometry)

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3b) Nitrites in urine

- Positive for **bacteriuria** → demonstrates the presence of bacteria that are able to convert nitrates (normally present in urine) to nitrites
- Proves mainly gram-negative bacteriuria (*Escherichia coli, Proteus, Klebsiella*)
- a positive result in the presence of these bacteria requires nitrates in the patient's diet and sufficient urine incubation (several hours) in the bladder, therefore it is necessary to test it from first-morning urine
- sensitivity is about above 100,000 CFU in 1 ml of urine

Determination of nitrites:

The reaction takes place in two steps:

I. diazotation – reaction of aromatic amine with nitrite formed from nitrates by bacterial activity

II. azokopulation – reaction of another aromatic amine with diazonium salt to form azo-dye

For example, NITRIPHAN *strips (Erba Lachema)* work on this principle

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3c) Leukocytes in urine

- Proof of **leukocytes** only complements microscopic examination.
  - Detection is based on the esterase reaction, which is positive even in the case of leukocyte decay.
- Almost exclusively neutrophil granulocytes and macrophages are determined. Lymphocytes do not provide a response.
- The limit of detection is 10 to 25 leukocytes in 1 µl of urine
- 20 leukocytes in 1µl of urine is already considered pathological **leukocyturia**.

3d) Proteins in urine

- **albumin** is most sensitively detected (in glomerular proteinuria), globulins are less sensitive and the result is completely negative for Bence- Jones protein (= paraprotein formed by light chains of monoclonal immunoglobulin. It occurs in some blood diseases, especially in myeloma).

Age category	Kids	Adolescent	Adults
Normal finding	37 mg/24 hrs	60 mg/24 hrs	60 mg/24 hrs
Maximum	max. 70 mg/24 hrs	max. 120 mg/24 hrs	133 mg/24 hrs
Pathological findings	nad 150 (200) mg/24 h		

- the protein detection in the urine must be verified by another method - a classic precipitation reaction with sulfosalicylic acid from a urine sample (from a 24 hours collection)
- false positive reaction: give **sulfonamides,** quinine and choline preparations present in the urine, **oral antidiabetics,** and higher concentrations of penicillin
- the sensitivity of the method is from 0.15 to 0.20 g/l
- **Proteinuria** could be **functional** or **pathological**

  - Functional (up to 150/200 mg protein/day)
  - Pathological (**over 150/200 mg** protein/day)

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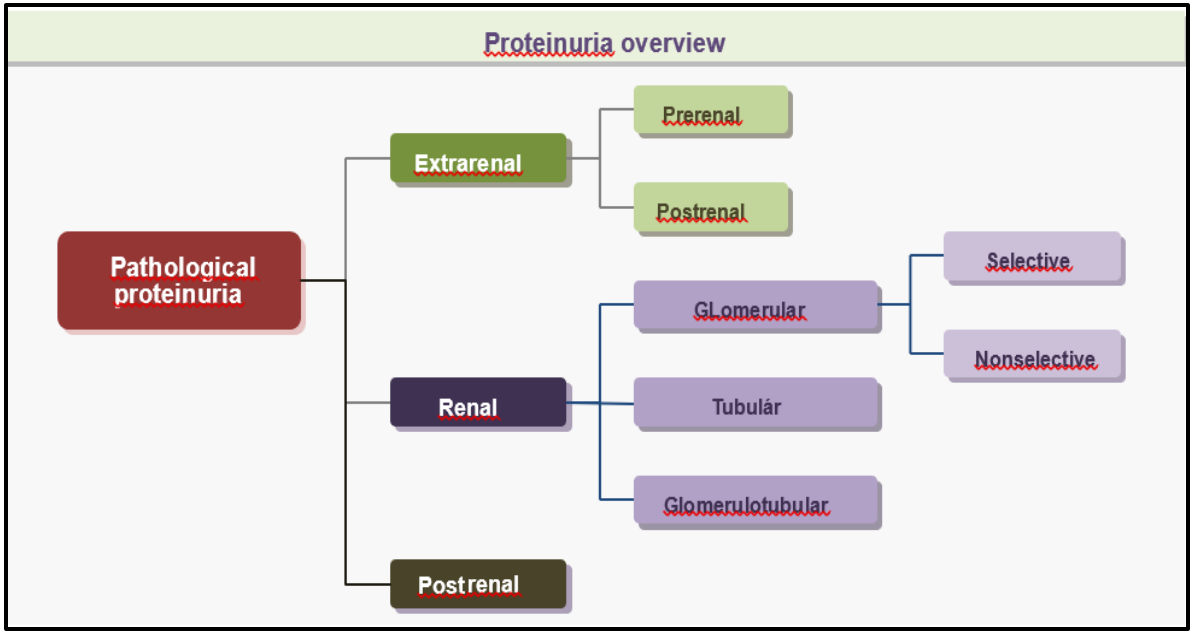
3d) Proteins in urine

- **Functional** proteinuria

- *orthostatic*: while standing;
- *transient:* change in renal hemodynamics (short-term): after exertion, emotional excitement, cold, stay at high temperature etc.;
- during *pregnancy*



- **Pathological** proteinuria : prerenal , renal, postrenal

1) Prerenal proteinuria : tubular resorption capacity is exceeded (the concentration of some protein in the serum is ↑ increased without a renal cause): causes: *myeloma* (Bence- Jones protein *)* ; *bronchial carcinoma; monomyelocytic leukemia*

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3d) Proteins in urine

Postrenal proteinuria : occurs when protiens get in the urine from the excretory urinary tract (proteinuria caused by blood or lymph transition, or infection, cell separation, and cytolysis)

3) Renal proteinuria

- ***Glomerular proteinuria :*** the best known, the protein concentration is usually high (≥ 1 g/l). Glomerular permeability for proteins is increased + tubular resorption capacity is exceeded. Depending on the degree of damage to the basement membrane, the following are distinguished:
  - ***selective****,* with less glomerular damage – in urine : proteins with a relative molecular weight of 65,000 – 130,000, e.g. *albumin* , *transferrin, orosomucoid*
  - ***non-selective***, with more severe damage to the glomerular membrane – in the urine get proteins with rmw of 65,000 – 1,000,000, i.e. *immunoglobulins*, *macro-globulin,* the filtrate protein composition is close to plasma
- **Tubular proteinuria:** weaker (<1 g/l) than glomerular, impaired reabsorption ability in the proximal tubule – secreted proteins have a relative molecular weight below 65,000

3e) Glucose in urine

- the sensitivity of the test is above 2 mmol /l
- the detection reaction for glucose is based on enzymatic oxidation by glucose oxidase (hydrogen peroxide is formed → peroxidase → colored product)
- **false positivity** appears in urine containing **oxidizing substances** (chloramine, persteril, peroxide)
- **false negative:** can occur in urine with a high concentration **of reducing** substances (ascorbic acid = vitamin C and other reducing substances, also drugs: salicylates, gentisic acid, DOPA) and bilirubin above 60 mg/l
- it is important mainly for detecting diabetes and some renal tubular disorders
- There can be various sugars in the urine, the most important → glucose
- ***Glucose*** is practically completely absorbed from primary urine, where it is in the same concentration as in plasma, up to 0.1%, which corresponds to a waste of 1.1 µ mol /24 hours (approx. 200 mg/24 hours) X the capacity of tubular resorption is limited

3e) Glucose in urine

The origin of **glycosuria** can be *prerenal* or from the kidneys *(renal)*

- ***prerenal glycosuria*** may be
  - *temporary* (cause: alimentary or infusion, emotional stress, etc.) or
  - *permanent :* cause: diabetes mellitus
- ***renal glycosuria:*** it is the proximal tubule resorption disorder (there is a normal glucose level in the blood)

Differentiation between ***prerenal and renal glycosuria*** can be done, for example, by simultaneous determination of glucose in the blood and in the urine.

3f) Ketone bodies in urine

- Ketone bodies include: ***acetone*** (the only keto compound !); ***acetoacetic*** acid, and ***β - hydroxybutyric*** *acid*
- The source of these acids are ketoplastic AA and the breakdown of FA in the liver.
- Besides acetone (the end product), ketone bodies are further metabolized in peripheral organs. They are an important energy source for the brain,which cannot break-down free fatty acids and its only nutrient is glucose, or ketone bodies.

This urine determination method is more sensitive to acetoacetic acid than to acetone. *β* - hydroxybutyric acid is not detectable

↑ ***Increased ketone bodies in urine:***

- *Starvation*– reduction diet with carbohydrates restriction, ↓ carbohydrate intake due to excessive energy expenditure (febrile and cancer diseases – GIT esp., energy expenditure during sports), vomiting, diarrhea
- *Excess of proteins and especially fats in the diet* - with a relative lack of carbo-hydrates (athletes, reduction diets)
- *Poor parenteral nutrition*
- *Diabetes mellitus* - diabetic ketoacidosis with hyperglycemia, diabetic precoma and coma (inability of the body to use Glc); insulin overdose, etc.; ketoacidosis in diabetes is more severe than in starvation because the inhibitory effect of insulin on adipose tissue (inhibition of lipolysis) is absent.

3g) Blood in urine (erythrocytes, hemo and myoglobin)

- the test strip enables proof of free hemoglobin, or ERY in urine (bright blue color)
- the sensitivity of the reaction is 5-10 erythrocytes in 1 ul of urine
- a false positive reaction is usually in the presence of a more significant number of leukocytes, bacteria or fungi
- the prerenal reason for positivity is pathological conditions leading to the presence of hemoglobin and myoglobin in the plasma (hemolytic conditions, crush syndrome, burns, myopathy, etc.)

Erythrocyturia

Causes of erythrocyturia

- malignant tumors in up to 20% of cases
- urolithiasis (bladder stones)
- inflammation of the kidneys (erythrocyte cylinders)
- severe inflammation of the urinary tract (leukocyturia)

Hemoglobinuria

occurs due to rapid and excessive breakdown of erythrocytes in blood vessels (intravascular hemolysis).

Myoglobinuria

The cause of myoglobin presence in urine can be - excessive muscle exertion, injuries, and (especially) muscle necrosis, including myocard (myocardial infarction).

3h) Bile pigments (bilirubin)

Bile pigments in urine:

- *bilirubin* (oxidation to colored products, azocopulation)
- *urobilinogen,* stercobilinogen *(*Ehrlich's reagent: 4-dimethyl-amino-benzaldehyde in HCl)
- *urobilin, stercobilin* (Schlesinger experiment: suspension of zinc acetate in ethanol)

Bilirubin: is a heme degradation product produced mainly in the reticuloendothelial cells of the spleen and liver. It is an orange lipophilic substance. In blood, it usually circulates bound to albumin (unconjugated). In the liver cell, it conjugates with glucuronic acid (conjugated bilirubin). Glucuronides are excreted into bile → intestine → part is absorbed from the intestine into the bloodstream and thus undergoes the enterohepatic cycle.

Hyperbilirubinemia: increased value of conjugated bil. and also unconjugated bilirubin (positive reaction to bilirubin in urine is above 35 μmol/l)

- conjugated bilirubin: water-soluble; therefore, it is filtered in the kidneys and excreted in urine. Physiologically, it does not occur in urine.
- positivity: in **hepatopathies**, detectable concentrations of conjugated bilirubin in the urine appear in obstructive and hepatocellular **icteruses** (*bile stones, hepatitis, toxic liver damage, liver tumors*, etc.)

3h) Bile pigments (bilirubin, urobilinogen)

- Conjugated bilirubin produces an azocopulation reaction with a stabilized diazonium salt in the strip indicator field to produce a red to red-violet coloration.
- False negative results may be caused by a high concentration of ascorbic acid in the sample.
- Positive bilirubin findings in urine should be correlated with the determination of total and direct bilirubin in serum.
- The urine, tested for bilirubin, should not be exposed to direct sunlight for a longer period of time (oxidation of bilirubin and false negative result occur)

Tab. 1 Výskytu bilirubinu a urobilinogenu v moči u různých typů ikteru

Typické nálezy	Hemolytický ikterus	Hepatocelulární ikterus	Obstrukční ikterus
Bilirubin	neg.	+	+
Urobilinogen	+	+	neg.

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3h) Bile pigments (urobilinogen)

- **Urobilinogen (Ubg)** and **stercobilinogen** are the end products of hemoglobin catabolism. Analytical distinction between Ubg and stercobilinogen is difficult + dg has no significance → both substances are determined and summarized as urobilinogen .
- The bilirubin conversion to urobilinogen and stercobilinogen occurs partly in the bile ducts but mainly in the large intestine by the bacteria-reducing activity.
- Most of the urobilinogen is excreted in the feces, some undergo the entero-hepatic cycle, and a small amount is excreted by the kidneys in the urine.
- The amount of urobilinogen produced → proportional to the concentration of bilirubin excreted by bile into the intestine.

Urobilinogen:

- positivity is: in conditions associated with ↑ breakdown of hemoglobin, in limited liver parenchyma function (hepatitis, cirrhosis, tumors and toxic liver damage) and in increased production and resorption of Ubg during biliary tract infections, enterocolitis and ileus.
- some drugs give a false positive reaction
- a negative finding is in the case of complete obstruction of the bile ducts and the absence of normal bacterial intestinal flora

4) Examination of urine sediment (US) = Morphological examination of urine

- indication: clinical suspicion of kidney and urinary tract disease, nephrological or urological control (monitoring of the course of renal or urological disease)
- symptoms of kidney and urinary tract diseases mainly include the excretion of leukocytes , erythrocytes, and epithelial cells - we focus on the visual field in terms of the number of elements
- We distinguish 1. **Semi-quantitative** US examination (after centrifugation of urine under standard conditions). 2. **Quantitative US examination** according to Hamburger .

Ad.1 Semi-quantitative (approximately):

It must be done **within 1 hour** after urine collection or **get preserved** and processed within **3 hours**! Preservation does not protect cells from decay. For the exact number and type of cylinders, the urine must be processed within 30 minutes!

Normal finding-centrifuged urine: 1-2 Ery, 0-5 leukocytes in women and 0-1 Leu (men) per visual field. In a healthy individual → poor urinary sediment

Execution conditions

Spinning: 10 min/400-600g [R (g) = 1.117 . year n2. 10-5] 1)

Concentration: to 1/20 of the original volume, for quantitative determination to 1/10 of the original volume exactly

Microscopy : magnification 200x

Staining : so-called *supravital* staining (i.e. staining of cells just after their death)

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4) Examination of urine sediment (US)

- ***Ad 2: <u>Quantitative</u> US examination:***

– According to Hamburger, urine is collected for 3 hours, the collection time is given with an accuracy of minutes, and the entire volume of collected urine is measured and delivered to lab. The volume should not be less than 100 ml. Urine must be delivered to the laboratory as soon as possible after collection. In the quantitative examination of urine sediment according to Hamburger, *the number of elements per 1 minute* is evaluated: Erythrocytes up to 2000/min./ Leukocytes up to 4000/min. /Cylinders up to 60 - 70/min.

Močový sediment obsahuje

- neorgánové součásti (krystaly a amorfní soli)
- orgánové součásti (buňky)
- mikroby, kvasinky, plísně, parazity
- náhodné příměsi a znečištění

Močový sediment orgánový

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Evaluation of pathological findings of urinary sediment

• **1.** Increase of erythrocytes and hemoglobin (**erythrocyturia , hematuria**)

Hematuria: micro or macroscopic. According to origin: **glomerular, non-glomerular** (renal hematuria and from the urinary tract).

Add. ***Glomerular erythrocyturia*** : when Ery passes through the glomerular membrane, shape deformation occurs + a change in the structure of the erythrocyte membrane. Distinguishing between erythrocytes that have passed through the glomerular membrane and others → possible by examination of urine sediment in phase contrast. In chronic glomerulopathies, simultaneous assessment of the degree of erythrocyturia and proteinuria is important, especially for differential diagnostic assessment of the cause of glomerular damage.

Add. ***Nonglomerular erythrocyturia***

Renal: differential diagnosis: - consider bleeding from ruptured blood vessels in renal parenchyma tumors (most often Grawitz's tumor), renal cystosis, or renal tuberculosis

From the urinary tract: It is usually caused by stones, inflammatory congestion of mucous membranes, tumors and injuries

4) Examination of MS: elements, cylinders

- **2.** Leukocytes in urine **(leukocyturia)**
  - inflammatory diseases of the urinary tract, interstitial nephritis, malformations of the uropoietic system, urolithiasis,urinary tract and bladder tumors.
- **3. Epithelia**
  - from ***renal tubules:*** proliferation indicates tubular involvement
  - epithelial cells from ***other parts of the urogenital system*** : indicates an inflammatory process with more intensive desquamation of the epithelium, without the possibility of localization of the process.
  - ***squamous*** epithelial cells: they originate from the vagina and the urethra. The occurrence of squamous epithelial cc is most common in the women urine, it has no diagnostic significance.
- **4. The cylinders**
  - they arise exclusively from renal tubules as casts, the finding is clinically significant. The matrix of the cylinders is formed by the so-called Tamm's Horsfall protein (a mucoprotein secreted by tubular cells). We distinguish between ***hyaline, granular, wax, fat, and cellular*** (erythrocytic, leukocyte, epithelial) cylinders .

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4) Examination of MS: other elements

- other elements:

  bacteria, crystals, yeast, fungi, trichomonads , sometimes sperm, textile fibers, powder granules, etc. Estimation of bacteriuria intensity is only possible in completely fresh urine, in older urines there is a significant multiplication of bacteria. Yeast is mainly found in the urine of diabetics. Trichomonads can only be found in fresh urine as organisms with the typical whirling movement of the flagellum.

5. <u>Crystals:</u> they have different shapes → the composition is determined according to the shape (**urate, phosphate, oxalate** and others)

Urinary stones (urolithiasis)

Stones contain

- *organic matrix* (polysaccharides, proteins)
- *inorganic and other impregnating substances* (calcium oxalate, calcium and magnesium phosphate, carbonates, uric acid and its salts (urates), cystine, cholesterol)

A brief description of the urinary stone analysis procedure

1. Analysis of appearance
2. Qualitative and quantitative analytical reactions
3. Microscopic-chemical examination
4. Crystallography
5. Thermoanalysis
6. Spectral analysis etc.

Stone analysis is carried out at a specialized workplace. ↓ number of samples → it is an advantage if one lab processes samples for a larger area (region). One such company is the Brno company *Calculi.* Details about urolithiasis can be found, for example, on the website of the mentioned company Calculi : *http://www.calculi.cz/urolitiaza.php*

Case study 1

1. What would explain the apparent disagreement between the nitrite and leukocyte reaction?
2. What influences the clarity (or opacity) of the sample in the chemical examination?
3. What patologic condition could be the cause of this urinalysis?

Responses:

Ad 1.:

- Non-nitrate reducing organism (i.e., bacteria-enterococci, staphylococci, Acinetobacter, adenovirus, yeast, trichomonads, and chlamydia)
- or Trauma. (Other less likely possible.)

False-negative nitrite tests in urinary tract infections occur in cases with a low colony forming unit (CFU) count, or in recently voided or dilute urine

Ad 2. Large blood. (Also slightly enhanced protein)

Ad 3. Inflammation (urinary tract infection? – add more test, for example cultivation etc.)

Case 1

Physical Examination	**Observed Result**
Color	Light yellow and cloudy
Chemical Examination	**Observed Result**
Glucose	Neg
Bilirubin	Neg
Ketone	Moderate
Specific Gravity	1.015
Blood	Large
pH	5.0
Protein	30
Urobilinogen	0.2
Nitrite	Neg
Leukocytes	Moderate
Confirmatory Tests	
Protein (SSA)	Trace
Ketones (Acetest®)	Pos
Bilirubin (Ictotest®)	

Source:https://ascls.org/urinalysis-cases-and-critical-thinking/

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Case study 2

1. What could explain the single most unexpected finding within the chemical reactions?
2. What patologic condition could be the cause of this urinalysis?

Responses:

Ad 1.: Negative leukocytes could result from any or all three of the following. 1) Alkalinity 2) >3g/dL glucose 3) High specific gravity.

Ad 2. Inflammation (could be complication of diabetes, urinary infection)

Source:https://ascls.org/urinalysis-cases-and-critical-thinking/

Case 2

Physical Examination	**Observed Result**
Color	Yellow-brown and clear
Chemical Examination	**Observed Result**
Glucose	2000
Bilirubin	Small
Ketone	Neg
Specific Gravity	1.030
Blood	Moderate
pH	8.5
Protein	2000
Urobilinogen	0.2
Nitrite	Positive
Leukocytes	Negative
Confirmatory Tests	
Protein (SSA)	2+
Ketones (Acetest®)	
Bilirubin (Ictotest®)	Small

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Resources

- Stern P. and col.: General and clinical biochemistry, Karolinum , Prague, 2011
- Schneider P. and col.: Determination analytes in clinical biochemistry, Part I , Karolinum Prague 1999
- DRŠATA J., et al . *Selected chapters on pathobiochemistry Part II* . Prague: Karolinum , 2009, p.187. ISBN: 978-80-246-1715-2

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