

Examination of urinary sediment

Morphological components of urine can be detected by **microscopic examination of the urinary sediment** and more recently by flow cytometry.

Urine sediment analysis doesn't belong to screening procedure. It's used during these *indications*:

- in case of a positive finding of a chemical examination of the urine (positive erythrocytes, protein, nitrites);
- in case of positive leucocyte test with diagnostic strips;
- in clinical suspicion of kidney and urinary tract diseases;
- during follow up examination of patients with nephrological or urological diseases

Preliminary examination of the urinary sediment

Diagnostic test strips can indicatively detect the presence of erythrocytes, leucocytes or bacteriuria with indirect test on nitrite (tab. 1). If the test with strips is found positive, a more demanding microscopic examination must be performed. However, by using diagnostic test strips the unnecessary microscopic examination can be reduced. Also the strips are used when the cell elements break down due to low osmolality or high urine pH, when the sample is standing for a long time or at higher room temperature. They indicate the presence of disintegrated erythrocytes or leucocytes even though the finding of urine sediment is negative. Pseudoperoxidase activity of hemoglobin or the activity of leucocyte esterases persists for several hours after release from the cells.

Tab. 1 Introductory examination of urinary sediment using diagnostic strips

Diagnostic strips (reaction zone)	Microscopic equivalent
Blood (hemoglobin/erythrocytes)	Erythrocytes, erythrocyte cylinders
Leukocytes	Leukocytes, leukocyte cylinders
Protein	Hyaline, waxy, granular cylinders
Nitrite	Bacteria

Microscopic examination of urinary sediment

Microscopic examination procedure

Urine sample preparation

- For microscopic examination, is most suitable to take a sample of a middle flow of the first or second morning urine. Usually the second morning urine is recommended because the first morning urine contains broken or damaged cell elements. Also the significant urine osmolality reduction and alkaline pH decreases occurrence of formed elements due to their lysis.
- It is necessary to work with fresh urine within 1 hour after collection for examination of urinary sediment. By prolonging the interval between collection and examination, there is higher chance of cell damage and breakdown.
- The urine sample must be well mixed and then 5 to 10 ml of urine is measured into a test tube. It's centrifuged at 400g for 5 minutes, preferably at 4°C. Then 9 parts of supernatant are carefully removed which means the sediment is 10 times concentrated compared to the original urine. If we work with a stained sample (see below), we add a dye in an amount that corresponds to 10% of the total volume.

Staining procedure

1. Dilute 50 µl of the staining solution (alcian blue and pyronin B in a ratio of 1:1) in 0.5 ml of urine sediment and mix gently.
 2. After 5 minutes, transfer 13 µl of stained sediment on to the microscopic slide and cover with a coverslip 18x18 mm. A larger volume of stained sample with the appropriate size of coverslip can also be used.
- First, we view the sample starting with lower magnification of 100-200x so we can assess the distribution of the elements and notice rare particles such as cylinders and epithelial cells. Then we switch the magnification to 400x to count corpuscular elements in at least 10 randomly chosen visual fields. Higher accuracy of element counting is achieved by using a chamber (Bürker or other chamber).

Possibilities of microscopic examination

Bright-field light microscopy and phase contrast techniques are used for microscopic examination of urinary sediment, in special cases also microscopy with polarisation filter can be used.

1. Bright-field light microscopy

This method enables introductory orientation or identification of pathologic elements. Using bright-field light microscopy for viewing **preparation without staining** can lead to missing out some elements

such as hyaline casts and bacteria. Unstained preparations are harder to identify leucocytes, macrophages and renal tubular cells. Therefore using **supravital staining** is recommended for accurate morphological identification of urinary elements because it highlights some details of cellular structure. The term *supravital* means staining of wet unfixed preparation, in which some cells can still be alive. Nowadays most recommended is Sternheimer's stain, utilising colored contrast of blue and red provided by Alcian Blue and Pyronine B. The Alcian Blue stains the surface of cells and other elements due to his high affinity to mucopolysaccharides, while Pyronine B stain mostly cytosol by penetrating inside cells.

2. Phase contrast microscopy

It's suitable for rapid evaluation of unstained preparations. By using this method we get more detailed evaluation of sediment, especially better recognition of leucocytes, casts, crystals and differentiation of red blood cells including morphologic alterations of their membranes. Increasing the contrast enables a better display of details achieved by shifting the phase of the light wave of part of the rays.

3. Microscopy with polarisation filter

It is suitable for better identification of crystals and lipid bodies.

Components in urinary sediment and their evaluation

In the urinary sediment, we assess **organ components**, mainly represented by cell or cylinders and **non-organ components** among which we include crystals. Beside that, we try to notice the presence of microorganisms and various artifacts can occur. The main components of urinary sediment are summarized in Table 2.

Tab. 2 Overview of the main components of urinary sediment

Cellular elements	blood cells	erythrocytes
		leukocytes
		lymphocytes
		macrophages
	epithelia	renal tubular cells
		transitional epithelial cells
		squamous epithelial cells
	tumour cells	
Casts	cell-free	hyaline
		granular
		wax
		fatty
	cellular	erythrocytic
		leukocytic
		epithelial
		bacterial
Microorganisms	bacteria	
	yeast	
	trichomonades	
	mould	
Crystals		

Corpuscular elements

Leukocytes

- **Polymorphonuclear granulocytes** are the most frequently detected. They are round cells (average size 10 µm) with granular cytoplasm. The nucleus is segmented, but it often undergoes degenerative changes which leads to difficulty of distinguishing it from the cytoplasm. Sometimes it's poorly stained; if it does stain, it appears distinctly blue while the cytoplasm is red or red-brown. The appearance of granulocytes is also affected by urine osmolality. They often gather. The finding is characteristic for urinary tract infection, if erythrocytes are present at the same time, it may affect the glomeruli. In around 50% cases we can find leukocytes with bacteria. Eosinophils can be detected only by using special staining. A false positive finding may be caused by contaminated urine (vaginal secretion, failure to follow urine collection instructions - first stream).
- The occurrence of **lymphocytes** in the urine is mostly associated with chronic inflammation of the kidneys,

sometimes with viral infections and further with kidney rejection after transplantation. Lymphocytes have a homogenous nucleus with a thin cytoplasmic margin. The ratio of nucleus to cytoplasm and the smooth structure of the cytoplasm are best distinguished from renal tubular cells.

- Sometimes we can also find **macrophages**. Their finding is relatively common in urinary tract infections.

- **Reference values:**

- ≤ 10 leukocytes/ μl urine,
- approximately < 5 leukocytes/field of view.

Erythrocytes

- The presence of erythrocytes in the urine is usually a symptom of kidney or urinary tract disease. Erythrocytes are smaller than leukocytes. They appear as non-nuclear discoid bodies of an average size about $6\text{ }\mu\text{m}$. In hyperosmolar urine erythrocytes easily lose intracellular fluid, their diameter decreases and they become crenated to spiny. On the other hand, in hypoosmolar urine, fluid enters erythrocytes leading to enlarging of cells that may disintegrate. If their hemoglobin content is low, they are difficult to recognize and appear as erythrocyte *ghosts*.
- The appearance of urinary red blood cells can indicate their origin. If the glomerular membrane is significantly damaged, then it's possible not only for proteins but also erythrocytes to penetrate. As the erythrocytes pass through the glomerular membrane, the shape is deformed and the structure changes. Erythrocytes that show deviations from the discoid shape are called **dysmorphic**. Sometimes they can have the shape of *tires* (called as *ring or annular erythrocytes*), other times the membrane of erythrocytes extends into vesicles, in these cases we talk about acanthocytes. Increased occurrence of dysmorphic erythrocytes is typical for renal glomerular involvement. The *Zvýšený výskyt dysmorfických erytrocytů je typický pro poškození ledvinových glomerulů*. Significant proportion of dysmorphic erythrocytes is distinctive for affection of kidney glomeruli. If more than 80% of urinary erythrocytes are dysmorphic, it is conclusively a **glomerular hematuria**, simultaneously a proteinuria is found. If more than 80% of urinary erythrocytes are isomorphic (normal shape), it is a non-glomerular hematuria whose source is bleeding into urinary tract or bleeding from ruptured blood vessels in kidney tumors or urolithiasis. Phase contrast microscopy is required to identify dysmorphic erythrocytes.
- The causes of hematuria must always be clarified, especially cancer or severe glomerulopathy (glomerulonephritis) must be ruled out.
- The cause of increased number of erythrocytes in the urinary sediment can also be extreme physical exertion, the use of anticoagulants or the admixture of menstrual blood.

- **Reference values:**

- < 5 erythrocytes/ μl urine,
- approximately < 5 erythrocytes/visual field.

Epithelium

They come from the epithelial lining of the renal tubules and urinary tract.

Renal tubular cells

- Their occurrence in urinary sediment is always a pathological finding and indicates serious kidney damage, especially for diseases affecting the tubules (acute tubular necrosis, acute interstitial nephritis). They are relatively small cells (average size $13\text{ }\mu\text{m}$) only slightly larger than leukocytes, either round, irregularly polygonal, cubic or faceted with a smooth, usually eccentrically placed (dark blue in the colored sample) round nucleus, without nucleoli. They are characterized by a granular cytoplasm, which appears red in the stained example. They usually occur alone, sometimes they form clusters or casts.
- In unstained preparation, they are difficult to distinguish from transitional epithelial cells. Therefore sometimes referred to as "small round epithelial cells" in laboratory practice. They can also be mistaken for leukocytes.

Transitional epithelial cells

- They originate from the superficial or deeper layers of the transitional epithelium lining of the urinary tract. It is not possible to locate them in certain part of the urogenital tract. A common finding are surface layer cells that are round or ovoid with a round or ovoid nucleus located centrally or slightly eccentrically with a visible nucleolus and a cytoplasm that is usually granulated (less than tubular cells), the granulation is usually on the periphery of the cell, rarely around the core. The average size is around $30\text{ }\mu\text{m}$. Finding of these cells usually indicates an infection in the lower urinary tract, especially if leukocytes are also found. However, they can come even in urine of healthy individuals.
- Cells from deeper layers are smaller (average size $17\text{ }\mu\text{m}$), ovoid and their shape is much more variable (shape of clubs, hammers or cells with tails). Dual-nuclear cells are commonly found. We encounter them in the urine of patients with urothelial carcinomas or urinary stones.

Squamous epithelial cells

- They are the largest cells in the urinary sediment (average diameter $55\text{ }\mu\text{m}$), of rectangular or polygonal shape with small nuclei and abundant cytoplasm. Mostly, they come from urethra or vagina and their number depends on the quality of the urine sample collection. They are usually found in the urine from women if the urine sample is collected poorly, these samples have **no diagnostic significance**.

Tumour cells

- Tumour cells can be released into the urine during tumours of the kidneys, urinary tract and accessory organs (eg prostate). They are characterized by an irregular shape of the nucleus, which is usually significantly larger in shape in comparison to the cytoplasm. Without staining, the presence of tumour cells is difficult to detect (table 3).

Tab. 3 Morphologic features of cells in the urinary sediment

Cellular type	Nucleus	Cytoplasm
Erythrocyte	absent	discoid shape
Granulocyte	segmented, lobular, bright blue, sometimes poorly stained	granular, usually stained red
Macrophage	blue nuclei, often broken, non-homogenous chromatin	granular, usually includes erythrocytes or another phagocytosed particles
Lymphocyte	large, smooth, filling almost the whole cell	thin border of cytoplasm without granulations
Squamous epithelial cell	degenerated, small, polygonal, localised in the middle	abundant, not particularly stained
Superficial transitional epithelial cells	oval or round, usually localised in the cell centre, chromatin finely granulated, occasionally nucleolus visible	finely granulated cytoplasm, more at cell's periphery
Deep transitional epithelial cells	well defined, nucleoli visible	frequent granules, dark red of atypical shape
Renal tubular cell	homogenous bright, round or oval, usually not localised in the cell centre	roughly granular dense cytoplasm, often dark red, may contain lipid inclusions

Casts

Casts are structures of cylindrical shape formed in the distal tubules and collecting ducts of the kidneys. the matrix is made up of Tamm-Horsfall protein, which is produced by tubular epithelial cells protected by its surface. Under certain circumstances, such as low pH, high osmolality, high protein concentrations, Tamm-Horsfall protein can precipitate and form casts of tubules that are released into the urine and seen under microscope in a urinary sediment preparation. During the cast formation, various other components can be built in - for example leucocytes, erythrocytes, renal cells, pigments (hemoglobin, bilirubin), crystals and plasmatic proteins. The casts are the only elements in the urinary sediment that are **always from the urinary tract**. Morphology of casts depends on the shape of tubuli in which they have formed. If the tubulus where the cast originates is dilated due to atrophy or obstruction, the resulting casts are wide, typical of kidney failure.

According to their appearance, the cylinders are classified into:

- **cell-free**

hyaline,
granular,
wax,
fat;

- **cellular** (more than one third of casts surface consists of cells)

erythrocytic,
leukocytic,
epithelial,
bacterial.

Demonstration of cellular casts in the urinary sediment is indicative of pathologic process in the kidney.

Tab. 4 Overview and diagnostic significance of particular types of casts

Type of casts	Characteristics	Diagnostic significance
Hyaline	<ul style="list-style-type: none"> pure protein castings of tubules made of Tamm-Horsfall protein, they refract little light, not always absorb stain 	<ul style="list-style-type: none"> can occur in persons without renal disease eg. following unusual physical exercise, in fever or dehydration in fairly high number can indicate proteinuria
Granular	<ul style="list-style-type: none"> granules that are deposited in the form of drops in the hyaline matrix they originate as product of the breakdown of cells (tubular or blood) or proteins 	<ul style="list-style-type: none"> they occur in the patients with proteinuria or tubular cell damage pathognomonic for glomerular and tubular kidney diseases
Wax	<ul style="list-style-type: none"> they are formed from the originally granulated cylinder by the complete decay of the cell debris, so that it loses any internal structure the development of the wax cylinder takes several hours they have a homogenous structure, they are wide, with clearly broken ends, they strongly refract 	<ul style="list-style-type: none"> typical for patients with renal failure or renal insufficiency „casts of renal failure“ indicator of severe proteinuria
Fat casts and casts from fatty cells	<ul style="list-style-type: none"> surface bears fat bodies made of triacylglycerol or cholesterol in severe damage of glomerular membrane even the lipoproteins can enter filtrate; they are absorbed by tubular cells that degenerate, transform into fat bodies, and end entrapped in the cast matrix 	<ul style="list-style-type: none"> typical of glomerular damage in nephrotic syndrome
Epithelial	<ul style="list-style-type: none"> epithelium peeled from the renal tubules is trapped on the surface of hyaline matrix 	<ul style="list-style-type: none"> they occur in patients with tubular damage
Erythrocytes	<ul style="list-style-type: none"> erythrocytes are glued to the surface of the matrix erythrocyte degeneration can turn into hemoglobin cylinders 	<ul style="list-style-type: none"> indicates hematuria of renal origin, because cylinders are formed only in the renal tubules
Leukocyte (granulocyte)	<ul style="list-style-type: none"> mainly granulocytes are trapped on the surface of hyaline matrix 	<ul style="list-style-type: none"> their presence is typical for inflammatory kidney diseases of bacterial or non-bacterial origin evidence of renal leukocyte origin
Bacterial	<ul style="list-style-type: none"> clearly granular, very brittle 	<ul style="list-style-type: none"> are evidence of renal origin of bacteria very rare, as they require a large amount of bacteria in the kidney

Microorganisms

Bacteria

- Under physiological circumstances the urine contains bacteria in an amount of less than 10^5 /ml. They have appearance of small coccid or rod-shaped formations, which differ from other elements.
- The presence of bacteria can be a sign of non-sterile urine collection, as the bacteria multiply rapidly when the sample is allowed to stand for a long time.

Trichomonads

- They have a round or oval shape with whips, they are characterized by fast irregular movement when alive. Their frequent finding is in concomitant inflammation of the vagina.

Yeast

- They are slightly smaller than erythrocytes, oval but various sizes. We can find them in groups and sometimes grouped in the form of chains. They are common in diabetics, in patients treated with immunosuppressive drugs and sometimes after antibiotics.

Crystals

Examination of the crystals must be performed in the morning urine immediately after its collection. The finding of crystals, which occur relatively frequently in the urinary sediment, cannot be overestimated. The presence of crystals may be due to transient urinary supersaturation, eg when eating food rich in urates or oxalates, and is a

signal for increased fluid intake. Crystals form in vitro as the urine sample cools or the pH changes. The finding of crystals in these circumstances is clinically insignificant.

- Uric acid crystals and amorphous urates in **acidic urine and ammonium magnesium phosphate in alkaline urine** in urinary tract infections are common.
- Crystal detection is important in patients with urolithiasis. Their identification can indicate what kind of stones it is. However, it is not possible to conclude from the findings of crystals in the urine that there is a concrete of the same chemical composition in the urinary tract. Repeated detection of crystals is especially important in the control of patients after removal of the stone or in patients with recurrence of urolithiasis.
- Identification of hexagonal **cystine** crystals will support the diagnosis of cystinuria.
- The finding of ammonium magnesium phosphate crystals together with high urine pH indicates the probability of struvite stones.
- Flooding of **calcium oxalate crystals** is a characteristic finding in ethylene glycol poisoning, otherwise these crystals are a common finding especially in people with a higher intake of plant foods and are not related to the formation of stones. Another example is uric acid crystals in urate nephropathy.
- The presence of leucine and tyrosine crystals accompanies severe liver disease. Also, some drugs may be excreted in the form of crystals, especially in overdose, dehydration or hypoalbuminemia. It also affects the urine pH.
- **Cholesterol crystals** are a sign of severe glomerular membrane damage (tab. 5).

Tab. 5 Selected crystals in urinary sediment

Sort of crystal	Typical shape	pH of urine			Clinical significance
		Acid	Alkaline	Variable	
Urate	amorphous	+			<ul style="list-style-type: none"> ■ in healthy individuals
Uric acid	variable forms, "kegs", "rosettes"	+			<ul style="list-style-type: none"> ■ in healthy individuals ■ chemotherapy ■ gout
Ammonium urea	balls, „thorn apples“		+		<ul style="list-style-type: none"> ■ in healthy individuals ■ in old urine
Calcium carbonate	balls arranged in the shape of a dumbbell		+		<ul style="list-style-type: none"> ■ healthy individuals
Ammonium magnesium phosphate (triple phosphate)	coffin lid shape		+		<ul style="list-style-type: none"> ■ urinary tract infections ■ struvite concrements
Calcium oxalate	envelope (dihydrate), sponge-biscuit (monohydrate)			+	<ul style="list-style-type: none"> ■ in healthy individuals ■ intoxication with ethylene glycol
Cholesterol	flat plates with a corner broken off			+	<ul style="list-style-type: none"> ■ damage to glomerular membrane
Cystin	hexagonal prisms	+			<ul style="list-style-type: none"> ■ cystinuria
Tyrosine	thin pins in bundles or rosettes			+	<ul style="list-style-type: none"> ■ liver disease ■ aminoaciduria
Leucine	oil beads			+	<ul style="list-style-type: none"> ■ liver disease ■ aminoaciduria

Lipids

- Lipids can penetrate into urine through damaged glomerular membrane as plasmatic lipoproteins. Lipoprotein particles are larger than the proteins themselves and therefore lipiduria is associated with severe proteinuria

and signals severe kidney damage.

- Lipids occur in forms of loose isolated droplets or in clusters. Their presence may be caused by contamination, such as suppositories. Intracellularly localized fat inclusions are a sign of degenerative cell changes. Another form is cholesterol crystals or fat cylinders.

Other findings

- In the urinary sediment, we can notice motile spermatozoa with a long thin flagellum, mucus and fibrin fibers and various contaminating formations, such as fibers of toilet paper or various textiles.

Quantitative examination according to Hamburger

In indicated cases, a quantitative examination of urinary sediment according to Hamburger can be performed, which is used to measure the speed of excretion of erythrocytes, leukocytes and casts into urine. A patient collects urine for three hours. In collection period utmost deviation ± 30 minutes can be tolerated, and must be taken into consideration for calculation. When the urine collection is over, the samples must be delivered to the laboratory within one hour. At the same time, it is necessary to state the collection time to the nearest minute. The number of erythrocytes, leukocytes and cylinders in the 5 large squares of the Bürker chamber is evaluated in the sediment.

Reference values

- Erythrocytes up to 2000/min, tj. 33 Er/s.
- Leukocytes up to 4000/min, tj. 67 Leu/s.
- Cylinders up to 60–70/min, tj. 1 cylinder/s.

Automatic examination of urinary sediment

Devices for automated examination of urinary sediment are currently available. They are based on the **flow cytometry** or **digital imaging of particles**.

Flow cytometry

Flow cytometry is laboratory technique which enables to measure wide array of parameters in huge number of particles. In addition to hematology, its application in examination of urinary sediment is gradually expanding, which is used to hinder the laboratories as well because of the subjective error. In flow cytometry, the particles are labeled with different fluorophores and the cell suspension is driven through a narrow capillary afterwards. As they pass through the capillary, the particles encounter a beam of light, usually from a laser, which excites the fluorescence of the fluorophores. The laser light is scattered by the cell. The most frequently measured parameters are **light scattering at a small angle**, which is directly proportional to the size of the cells - the so called *forward scatter*, **light scattering to a large angle**, so called *side scatter*, which provides information about the internal structure of particles, and fluorescence of different wavelengths. The flow cytometry is a fully automated analyzer for the analysis and identification of cells and other elements of native urine samples.

Analysis procedure

- During examining urinary sediment by flow cytometry, urine is aspirated (0,8 ml) after mixing, diluted, and conductivity is measured.
- This is followed by automatic staining of the urinary elements with two different fluorescent dyes. The phenanthridine dye stains nucleic acids (orange fluorescence). The second dye is carbocyanine which is indicated for staining of negatively charged cell membranes, nuclear membranes and mitochondria (green fluorescence).
- The stained particles pass through the capillary and are irradiated with a laser beam, which is both scattered by the cell and excited by the fluorescence of the fluorophores. At the same time, the electrical conductivity of the particles in the capillary is measured. Identification and counting of elements is possible by evaluating the fluorescence of both dyes together with measuring the scattering of the radiation emitted by the laser and the measured conductivity.

All cell elements - erythrocytes, leukocytes, bacteria and epithelial cells - can be diagnosed using a flow cytometer. It is also able to differentiate some clinically significant modifications such as isomorphic and dysmorphic erythrocytes. It provides information on the presence of pathological cylinders, which however needs further microscopic examination. It also demonstrates crystalline structures, but does not distinguish between different types of crystals, which again must be specified by microscopic examination. The cytometer cannot detect trichomonads. Number of erythrocytes, leukocytes, bacteria, flat epithelia and casts is reported as number of elements/ μl . In order to increase accuracy using the diagnostic strips evaluated with reflection photometer. Agreement of flow cytometry with microscopy is reportedly 80–90%, and with diagnostic strips 72–96%. Flow cytometry significantly lowers the requirements for microscopic analyses, improves accuracy of the examination and facilitates standardisation of results.

Digital imaging of particles

In this method of automatic urine sediment analysis, a sample of centrifuged urine is injected into a planar cuvette. Particles present in the urine are scanned multiple times using a digital camera and their images are compared based on their size, shape and structure with a database that is part of the device software.

You can watch the video for automatic urine analysis here (<http://portal.med.muni.cz/clanek-10-automaticka-analyza-moce.html>).

Urine test reference values

Chemical examination

- pH 5–7.
- Relative density 1,016–1,022.
- Protein up to 0,3 g/l.
- Glukose negative.
- Ketone bodies negative.
- Bilirubin negative.
- Urobilinogen 3,2–16 µmol/l.
- Blood up to 5/µl.
- Leukocytes up to 10/µl.
- Nitrite negative.

Sediment (quantitative)

- Erythrocytes < 33/s.
- Leukocytes < 67/s.
- Casts < 1/s, only hyaline.

Links

Related articles

- Interpretace nálezů v močovém sedimentu
- https://www.wikiskripta.eu/w/Vy%C5%A1et%C5%99en%C3%AD_mo%C4%8Di
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