Urine analysis II: Physical examination
Urinary sediment

General Medicine

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2018/2019
The basic examination of urine includes:

- physical examination,
- chemical examination,
- morphological examination (urinary sediment).

**Physical examination of urine**

The physical urine examination consists of assessment of urine color, its smell, foam and turbidity; and also measurements of its pH, relative specific gravity and osmolality. For functional examinations, estimation of volume of urine per defined period of time is necessary.

**Volume of urine**

The daily volume of urine (diuresis) is markedly affected by drinking and food intake. Volumes less than 500 ml/24 hours or more than 2,500 ml/24 hours are considered pathological.

**Oliguria and anuria**

Oliguria is a term for urine volume < 400ml/24 hours; while anuria stands for volume < 100ml/24 hours.

Oliguria and anuria are basic symptoms of kidney failure. One of the causes might be dehydration resulting from insufficient water intake, or its excessive loss (diarrhoea, sweating). A low diuresis can happen also due to water retention (oedemas, transsudates in body cavities); or the cause may lie in primary damage to the renal parenchyma.

Oliguria/anuria can also result from a mechanical obstruction of the urinary tract (prostatic hypertrophy, wedged concrement, tumors in pelvic area). If the obstacle is located below the urinary bladder, we refer to the condition as retention of urine.

**Polyuria**

Polyuria denotes increase of daily diuresis above 2,500 ml.

Two types of polyuric conditions can be distinguished:

- Polyuria caused by water diuresis

  Water diuresis results from decreased tubular reabsorption of water in distal part of the nephrone. The tubular absorption as well as excretion of osmotically active substances are normal. Osmolality of urine is lower than osmolality of serum; and is always below 250 mmol/kg H₂O. The water diuresis comes physiologically as a result of high water intake; pathologically e.g. due to impaired secretion of adiuretin (diabetes insipidus).

- Polyuria caused by osmotic diuresis

  It results from either increased filtration of osmotically active substances due to their high concentration in the ECT (e.g. hyperglycemia), or from their decreased tubular absorption. The unabsorbed osmotically active substances “drag” water, leading in decrease in water reabsorption. Osmolality of urine is higher than 250 mmol/kg H₂O. The osmotic diuresis is characteristic e.g. for diabetes mellitus (glucosuria), polyuric phase of renal failure, or comes as an effect of diuretic drugs.

**Color of urine**

Fresh urine has an amber yellow color due to presence of yellow urochrome and pink uroerythrine. The color intensity depends on concentration and amount of urine that in turn depends on water intake and its extrarenal output. The early morning urine is usually more
concentrated and hence darker than later samples. Some pathological conditions or intake of certain exogenous substances (e.g. beetroot, rhubarb) change color of urine. Selected examples of characteristic changes in urine color are in table 1.

Table 1: Characteristic changes in urine color

<table>
<thead>
<tr>
<th>Color</th>
<th>Causing substance</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow to colorless</td>
<td></td>
<td>• increased diuresis in excessive drinking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• diuretic drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• diabetes insipidus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• polyuric phase of renal failure</td>
</tr>
<tr>
<td>Brown</td>
<td>bilirubin</td>
<td>• diseases of liver and biliary tract</td>
</tr>
<tr>
<td>Green-brown</td>
<td>biliverdin (originates from bilirubin by oxidation on air) – old urine</td>
<td>• diseases of liver and biliary tract</td>
</tr>
<tr>
<td>Yellow-orange</td>
<td>riboflavin, caroteness</td>
<td>• exogenous intake</td>
</tr>
<tr>
<td>Meat red (without turbidity)</td>
<td>hemoglobin</td>
<td>• intravascular hemolysis</td>
</tr>
<tr>
<td></td>
<td>myoglobin</td>
<td>• burns</td>
</tr>
<tr>
<td></td>
<td>porphyrins</td>
<td>• necrosis of muscles</td>
</tr>
<tr>
<td></td>
<td>beetroot</td>
<td>• inflammation of muscles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• porphyrias</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• exogenous intake</td>
</tr>
<tr>
<td>Meat red (with turbidity)</td>
<td>blood in urine - macroscopic hematuria</td>
<td>• diseases of kidney and urinary tract</td>
</tr>
<tr>
<td></td>
<td>(microscopic hematuria, which is demonstrable only by chemical or microscopic examination, does not affect color of urine)</td>
<td>• disorders of hemostasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• bleeding to urinary tract</td>
</tr>
<tr>
<td>Dark brown (turns black upon standing on air)</td>
<td>melanin homogentisic acid</td>
<td>• melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• alkaptonuria</td>
</tr>
<tr>
<td>Light red</td>
<td>urates</td>
<td>• hyperuricosuria</td>
</tr>
</tbody>
</table>

Smell of urine

The smell of urine must be evaluated with fresh samples as longer standing of urine on light leads to decomposition of some of its constituents and the odor changes. Certain disease states bring about characteristic smell of urine (table 2).
Table 2: Smell of urine

<table>
<thead>
<tr>
<th>Smell</th>
<th>Cause</th>
<th>Occurrence</th>
</tr>
</thead>
</table>
| Ammonia                   | presence of bacteria producing urease, an enzyme catalysing decomposition of urea to ammonia and water | • old urine sample  
                            |                                                        | • infections of urinary tract  
                            |                                                        | • diseases with chronic urinary tract retention (e.g. adenoma of prostate) |
| Acetone (overripe apples) | excretion of acetone in ketoacidosis                                  | • diabetes mellitus  
                            |                                                        | • starvation                                                      |
| Maple syrup or ‘maggi’ spice | branched chain carboxylic oxoacids (especially 2-oxoisocapronic, 2-oxoisovaleric acids) | • leucinosis (maple syrup disease) |
| Hydrogen sulfide, or even putrescent | bacterial decomposition of proteins releases H$_2$S from sulfur-containing amino acids | • infections of urinary tract  
                            |                                                        | • associated with proteinuria  
                            |                                                        | • cystinuria                                                      |
| Mouse                     | phenylacetate                                                         | • phenylketonuria                                 |

Foam of urine

Normal urine foams moderately, the foam is white and disappears quickly. More copious and rather permanent foam is found in proteinuria. In the presence of bilirubin the foam of urine is colored yellow to yellowish-brown.

Turbidity of urine

Fresh urine is usually without any turbidity. Cloudiness that develops after a long standing of a urinary sample is caused by epithelial cells; and is without pathological significance. Turbidity of fresh urine can occur due to presence of bacteria, leukocytes, lipids, phosphates, carbonates, uric acid, leucine, tyrosine, and oxalates. Chemical or microscopic examination of urine can differentiate among these causes of turbidity.

Relative specific gravity

Relative specific gravity (formerly relative density) is determined by mass concentrations of all dissolved substances excreted into urine.$^1$ Unlike osmolality it depends on the number of dissolved particles as well as on their molecular masses. High-molecular-weight compounds affect density more than electrolytes. In case of marked glucosuria or proteinuria the relative specific gravity of urine increases. Protein concentration of 10 g/l adds 0.003 to the urinary relative specific gravity; glucose concentration 10 g/l adds 0.004. The relative specific gravity of urine also significantly depends on temperature.

Under physiological condition the specific gravity of urine during 24 hours ranges from 1.015 to 1.025. In dilution and concentration tests the limit values obtainable are 1.003, and 1.040, respectively.

$^1$Relative specific gravity means ratio of specific gravity of urine to the specific gravity of water, which for practical purposes can be considered equal to 1. Difference between specific and relative specific gravity of urine, therefore, is negligible.

Specific gravity in the SI system has units kg m$^{-3}$. Specific gravity of a substance related to the specific gravity of water is a relative variable that has no units.
Ordinarily, the higher is the volume of urine, the lower is its specific gravity (diluted urine); and vice versa, i.e. in low diuresis the specific gravity of urine increases. But, in diabetes mellitus a high volume of urine with a high specific gravity is produced.

The specific gravity enables assessment of concentration ability of the kidneys. Values above 1.020 indicate good renal function and ability of kidney to excrete excess of solutes. Highly concentrated urine suggests a substantial decrease in the circulating blood volume.

Inability of kidney to concentrate urine is called hyposthenuria. The patient needs more water to excrete the same amount of solutes. Extremely diluted urine can be a sign of impaired kidney concentration ability, such as in cases of diabetes insipidus (lack of ADH), or side effects of some drugs. Combination of hyposthenuria with polyuria indicates damage to the renal tubular system with relatively intact glomerular filtration. A serious sign of kidney damage is isosthenuria. The kidneys lose any ability to concentrate or dilute; and excrete urine of the same specific gravity as the glomerular filtrate. The relative specific gravity remains permanently rather low, around 1.010. Simultaneous finding of isosthenuria and oliguria indicates a severe renal insufficiency. Elevation of the urinary relative specific gravity – hypersthenuria – results from proteinuria or glycosuria. Some changes in the relative specific gravity are listed in table 3.

**Table 3: Changes in relative specific gravity of urine**

<table>
<thead>
<tr>
<th>Term</th>
<th>Value of relative specific gravity</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eusthenuria</td>
<td>1.020 – 1.040</td>
<td>dehydration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glucosuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>proteinuria</td>
</tr>
<tr>
<td>Hypersthenuria</td>
<td>↑ 1.040</td>
<td>diabetes insipidus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hyperhydration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>renal failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diuretic drugs</td>
</tr>
<tr>
<td>Hyposthenuria</td>
<td>↓ 1.020</td>
<td>severe kidney damage</td>
</tr>
<tr>
<td>Isosthenuria</td>
<td>= 1.010</td>
<td></td>
</tr>
</tbody>
</table>

**Estimation of the relative specific gravity of urine**

For the estimation of the relative specific gravity of urine an urinometer, refractometer, or test strips can be employed.

- **Urinometer**
  Urinometer is a simple device for estimation of the specific gravity of urine. The widely used urinometers are calibrated for temperature of 15°C, because this temperature roughly corresponds to the temperature of urine standing one hour at room temperature. When used at different temperature, the values must be corrected: for every 3 °C above the calibrated temperature 0.001 is added, and vice versa. A urine sample of volume at least 10 – 15 ml is needed for this examination.

- **Refractometer**
  Refractometer measures the urine density on the basis of the index of light refraction. Compared to the urinometer, it offers several advantages: only 1-2 drops of urine suffice for the examination; and no correction for temperature is needed.

- **Diagnostic strips**
  The strip indication zone contains a suitable *polyelectrolyte acting as an ion exchanger, and bromthymol blue as an acid-base indicator*. The diagnostic strip function is based on the exchange of urinary cations, especially Na⁺, K⁺, and NH₄⁺, for ions H⁺ of the polyelectrolyte in the indication
zone. The released H\(^+\) acidifies weakly buffered acid-base indicator, originally in alkaline (unprotonated) form. Acidification changes the colour of the bromthymol blue. A disadvantage of this system is that the strips are insensitive to presence of urinary substances non-electrolytic in nature, such as glucose, proteins, urea, creatinine, and some others.

**Osmolality of urine**

Osmolality of urine depends on the *amount of osmotically active particles excreted into urine*, regardless of their mass, size and electric charge. The osmolality is expressed in mmol/kg. It is only loosely proportional to the specific gravity of urine. Measurement of osmolality is considered more accurate compared to the specific gravity and therefore favored.

Comparing both parameters, it can be stated that osmolality reflects the molar concentrations of all dissolved substances, whereas the specific gravity is related to their mass concentrations. Therefore, the osmolality will be much affected by changes in concentrations of low-molecular-weight substances such as Na\(^+\), glucose and urea. On the other hand, presence of protein in urine will affect predominantly the specific gravity.

Normal values of urinary osmolality under condition of ordinary water intake are 300-900 mmol/kg. Osmolality of urine depends on the dilution and concentration ability of the kidney. The extreme values in maximal dilution, or maximal concentration are 50, and 1200 mmol/kg, respectively. If osmolality of urine roughly equals osmolality of blood, it is *isosmolar urine*. Similarly, the *hypoosmolar urine* has osmolality lower than blood, i.e., below 290 mmol/kg; while *hyperosmolar urine* denotes urine of osmolality higher than blood.

Theoretically, it can be conceived that the final urine is formed from an isosmolar glomerular filtrate, to which the renal tubuli either add, or take clear, so called *solute-free water*.

The amount of the solute-free water can be determined as its clearance. In order to explain this term, let’s define first a **clearance of osmotically active substances (osmolal clearance)**:

\[
Cl_{\text{osm}} = \frac{U_{\text{osm}} \cdot V}{P_{\text{osm}}},
\]

Where
- \(Cl_{\text{osm}}\) = osmolal clearance in ml/s
- \(V\) = volume of urine in ml/s
- \(U_{\text{osm}}\) = osmolal concentration of urine in mmol/kg of water
- \(P_{\text{osm}}\) = osmolal concentration of blood plasma in mmol/kg of water

If the primitive urine has the same osmolality as plasma and the contribution of proteins to plasma osmolality is neglected, the volume of filtered primitive urine must be equal to the clearance of osmotically active substances \(Cl_{\text{osm}}\).

The **clearance of solute-free water** is the difference between the actual volume of the final urine excreted per unit of time, and the osmolal clearance:

\[
Cl_{\text{H2O}} = V – Cl_{\text{osm}}
\]

Where
- \(Cl_{\text{H2O}}\) = clearance of solute-free water in ml/s
- \(Cl_{\text{osm}}\) = osmolal clearance in ml/s
- \(V\) = volume of urine in ml/s

If the clearance of solute-free water is negative, it means that some of the solute-free water has been absorbed from the primitive urine, so that the final urine is osmotically more concentrated. On the other hand, a positive clearance of solute-free water means that a hypoosmolar urine is produced, in comparison to blood plasma diluted with the solute-free water. Physiological values range between \(-0.027\) and \(-0.007\) ml/s.
The kidney is able to excrete a high amount of solute-free water in order to prevent hyponatremia. In contrast, when water is scarce, its excretion is limited and the solutes leave the body in small amount of water.

**Examination of kidney concentration ability**

A disorder in kidney concentration ability is one of the first signs of renal diseases. It is examined as follows:

- **First**, *osmolality of an early morning urine sample* is measured. Value of osmolality over 600 mmol/kg indicates a good concentration ability and further testing is unnecessary.
- **Adiuretin test** reflects an ability of the distal renal tubulus and collection ductule to respond to adiuretin (vasopressin) by production of concentrated urine. A patient after an overnight liquid withdrawal receives to each nostril 10 µg (2 drops) of 1-deamino-8-D-arginine vasopressin (DDAV), which is a synthetic analogue of vasopressin with enhanced antidiuretic action and suppressed other pharmacological effects. The patient then collects urine in four one-hour intervals; and osmolality of each sample is determined. If in any of the samples the osmolality exceeds the reference value (table 4), it means a good concentration ability and the test can be terminated. Blood samples are taken simultaneously with urine, and used for estimation of serum osmolality. Values of urine and serum osmolality are then used for calculation of osmotic index ($U_{\text{osm}}/S_{\text{osm}}$), which provides more accurate information on the renal concentration ability than urine osmolality alone.

**Table 4: Physiological values of urine osmolality and osmotic index following adiuretin administration**

<table>
<thead>
<tr>
<th>Age</th>
<th>$U_{\text{mmol/kg H}_2\text{O}}$</th>
<th>$U_{\text{osm}}/S_{\text{osm}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20</td>
<td>970</td>
<td>3.34</td>
</tr>
<tr>
<td>21-50</td>
<td>940</td>
<td>3.24</td>
</tr>
<tr>
<td>51-60</td>
<td>830</td>
<td>2.86</td>
</tr>
<tr>
<td>61-70</td>
<td>790</td>
<td>2.72</td>
</tr>
<tr>
<td>71-80</td>
<td>780</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Another possibility is evaluation of urine osmolality under condition of liquid withdrawal of various length. It is seldom used nowadays.

The kidney concentration ability is impaired mainly in diseases affecting renal tubuli and interstitium, in which the medullar concentration gradient is disturbed. Next, it is decreased e.g. in diabetes insipidus.

It is possible to examine also the *dilution ability of the kidney* following intake of high volume of liquid. The test reflects renal ability to produce urine of osmolality lower than that of serum.
Estimation of osmolality

- **Osmometer**

For accurate measurement of osmolality the osmometers are used. These devices are based on the fact that dissolved substances lead to modification of some solvent properties, such as:
- decreased freezing point (cryoscopic principle),
- increased boiling point of solution (ebulioscopic principle),
- decreased pressure of solvent vapors above the solution.

Changes of these variables depend on concentration of osmotically active substances and can be measured by the osmometers with a high precision. Typically, a decrease in freezing point is measured. One mole of particles dissolved in 1 kg of water renders its freezing point 1.86 °C lower.

- **Approximately by calculation based on molar concentrations of Na⁺, K⁺, NH₄⁺ and urea in urine:**

\[
\text{Osmolality of urine} = 2([\text{Na}^+] + [\text{K}^+] + [\text{NH}_4^+] + [\text{urea}])
\]

This calculation fails if the urine contains a high concentration of other substances that are physiologically present only in traces, such as in a marked glycosuria or ketonuria.

- **Approximately by calculation from relative specific gravity:**

Procedure for urine not containing protein or sugar:
- last two digits from the value of relative gravity are multiplied by a factor 33
- example: relative gravity of urine = 1.019 \( \rightarrow \) 19 x 33 = 627 mmol/kg

Procedure for urine containing protein or sugar:
- the value of relative gravity is first corrected
  - in presence of protein, from the specific gravity 0.003 is subtracted for each 10 g/l of protein
  - in presence of glucose from the specific gravity 0.004 is subtracted for each 10 g/l of glucose
- last two digits from the corrected value of relative gravity are then multiplied by a factor 33

**pH of urine**

Kidney is organ where regulation of acid-base balance by excretion (or retention) of H⁺ takes place. The pH of glomerular filtrate is initially the same as in plasma. It is subsequently acidified during passage through the renal tubular system. In the proximal tubulus hydrogen carbonates are absorbed while H⁺ is excreted. In the distal parts of the nephrone K⁺ is exchanged for H⁺ and H⁺ is secreted by a proton pump.

Concentration of free H⁺ in urine is negligible in comparison to other ions; therefore, we can say that H⁺ is eliminated by the kidney in two forms:
- bound to present anions, e.g. phosphates (conversion of secondary phosphate to primary):
  \[
  \text{H}^+ + \text{HPO}_4^{2-} \rightarrow \text{H}_2\text{PO}_4^-
  \]
  or anions of some organic acids. This portion is designated as **titratable acidity**; under normal condition it represents 10-30 mmol/24 hours. It can be determined by titration of urine with sodium hydroxide (0.1 mol/l) to pH 7.4.
Physical analysis of urine. Urinary sediment

- as ammonium cation, which constitutes the most significant system.

\[
\text{H}^+ + \text{NH}_3 \rightarrow \text{NH}_4^+
\]

The amount of \(\text{NH}_4\) that is daily excreted into urine is 30-50 mmol/24 hours.

**Normal pH** of urine ranges from 5.0 to 6.5, the extreme values are 4.5 – 8.0. Extremely acidic or alkaline values are suspect of incorrect collection of urine samples.

pH of urine depends (table 5):

- **on food composition**
  In healthy person the urinary pH is mostly influenced by alimentation. A lactovegetarian diet alkalises urine. In contrast, diet rich in protein (meat) causes acidification of urine.

- **on acid-base balance**
  Under pathological conditions the pH of urine reflects disorders in acid-base balance. Changes in urinary pH follow compensation and correction processes in the kidney. Aciduria results from correction and compensation of metabolic and respiratory acidosis, while alkaliuria appears at the beginning of compensation and correction of respiratory and metabolic alkalosis. Excretion of acidic urine in acidosis and alkaline in alkalosis, however, occurs only in mild disorders and intact kidney function. Simultaneous findings of aciduria and ketonuria suggest starvation. Combination of aciduria, ketonuria and glycosuria is found in decompensation of diabetes mellitus.

<table>
<thead>
<tr>
<th><strong>Table 5: Common factors affecting pH of urine</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidic pH</strong></td>
</tr>
<tr>
<td>protein-rich diet</td>
</tr>
<tr>
<td>dehydration</td>
</tr>
<tr>
<td>diabetic ketoacidosis</td>
</tr>
<tr>
<td>metabolic and respiratory acidosis</td>
</tr>
<tr>
<td>starvation</td>
</tr>
</tbody>
</table>

Permanently alkaline pH of urine can signal:

- **Infections of kidney or urinary tract** by bacteria that produce enzyme urease. Enzymatic hydrolysis of urea yields ammonia, which alkalises urine. Similar situation, however, comes in an old and contaminated urine sample where bacteria proliferation occurred during sample storage.

\[
\text{H}_2\text{N-CO-NH}_2 + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^-
\]

- **Renal tubular acidosis, distal type.** It is a disorder of renal tubular cells in which the distal tubulus is unable to excrete protons.

The examination of urinary pH is most useful in **diagnostics of and treatment of urinary infection and urolithiasis**. Permanent alterations of urinary pH can contribute to formation of urinary concrements.

In **acidic urine** the concrements usually contain calcium oxalate; they can also readily originate from uric acid. Alkalisation of urine to pH above 7.0 can achieve gradual dissolution of the concrements from uric acids and prevent their further formation. Acidic pH also favors precipitation of cystine.

In **alkaline urine** the stones are made of phosphates. If pH is above 7.0, ammonium-magnesium phosphate (struvite – \(\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}\)), calcium phosphate, and calcium carbonate precipitate from the solutions.
Estimation of pH of urine

The urinary pH must always be estimated in fresh urine.

- pH in urine is routinely estimated with **diagnostic strips**. The indication zone for pH contains a mixture of suitable acid-base indicators. Typically, two compounds are used – **methyl red** and **bromthymol blue**, ensuring detection of pH in the range 5 – 9 by colour change from orange (acid pH) to green or even blue (alkaline pH). pH reading is possible with accuracy of 0.5 pH units.
- for precise measurement of urinary pH, **pH-meters** are used.

Examination of acidification ability of the kidney

The basic examination aiming at assessment of renal acidification ability is **measurement of pH in an early morning sample of urine**. It must be done immediately after sample collection; and usage of **pH-meter** is recommended. A healthy adult has pH of the early morning urine below 6.0. Higher value is suspect of acidification ability disorder and if there are no contraindications (e.g., severe restriction of kidney function), an **acidification test** after a load of NH₄Cl or CaCl₂ (in patients with impaired liver function) should be performed. Patient is given ammonium chloride (2 mmol per kg of body weight); three hours later a collection of urine in 3 one-hour intervals follows. Immediately after collection of each sample, its pH is measured. In intact renal acidification ability the pH of urine during such test should follow below 5.5.

Acidification ability is disturbed in patients with the distal type of renal tubular acidosis, caused by inability of distal tubulus to excrete H⁺.

In cases the acidification test is not informative; **alkalisation ability** of the kidney is examined following oral or intravenous administration of sodium hydrogen carbonate.
Examination of urinary sediment

Analysis of urinary sediment is not considered as a routine screening test. It is performed in the following indications:
- chemical analysis finds some pathological components in the urine, especially blood, protein, nitrite,
- the strip test for leukocytes is positive,
- clinical signs point to a disease of kidney or urinary tract,
- control examination of patients with nephrologic or urologic diseases.

Corpuscular elements in urine are investigated by microscopic examination of urine sediment, and nowadays also by flow cytometry.

Introductory examination

The test strips can detect erythrocytes, leukocytes and bacteriuria, the latter indirectly as presence of nitrite. Positive finding with the strip necessitates further microscopic examination. Therefore, the strips cannot replace the microscopy, their proper usage, nevertheless, can limit unjustified requests for more laborious microscopic examinations. The strips are useful even if the cellular elements decompose due to low osmolality or high pH of urine, due to long standing of the sample or high room temperature. The finding in the urinary sediment is negative, but still the presence of decomposed erythrocytes and/or leukocytes is revealed by the test strip. The pseudoperoxidase activity of hemoglobin or the activity of leukocytic esterases persists up to several hours following release from the cells.

Tab. 6 Introductory examination of urinary sediment with the diagnostic strips

<table>
<thead>
<tr>
<th>Diagnostic strip (reagent pad)</th>
<th>Equivalent in microscopic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (hemoglobin / erythrocytes)</td>
<td>Erythrocytes, erythrocytic casts</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Leukocytes, leukocytic casts</td>
</tr>
<tr>
<td>Protein</td>
<td>Casts granular, wax, hyalite</td>
</tr>
<tr>
<td>Nitrites</td>
<td>Bacteria</td>
</tr>
</tbody>
</table>

Microscopic examination

Sample preparation

The most suitable sample for microscopic examination of urinary sediment is the middle flow of the first or second morning urine. The second morning urine is recommended because in the first morning sample the cellular elements can be damaged or decomposed. Also a low osmolality of urine and alkaline pH in general decreases occurrence of corpuscular elements because of their lysis.

For examination of urinary sediment, it is necessary to process a fresh urine sample within one hour from the collection. The sample is mixed well and 5 or 10 ml of it is measured into a centrifugation test tube. It is centrifuged at 400 g for 5 minutes, at 4 °C at best. Then 9 parts of 10 of the supernatant is careful removed, which results in suspension of sediment 10-times
concentrated compared to the original urine. The sediment can then be viewed under microscope equipped with phase contrast, or subjected to staining before microscopic examination.

**Staining procedure:**
1. 50 µl of staining solution (Alcian Blue and Pyronine B in ratio 1:1) is combined with 0.5 ml of sediment suspension and gently mixed.
2. After five minutes 13 µl of stained sediment is transferred on the microscopic slide and covered with a coverslip 18 x 18 mm (higher volume of the sample can be taken with the corresponding size of the coverslip).

**Microscopic examination**
First, examination starts with lower magnification 100-200x, at which distribution of elements can be assessed and rare particles such as casts and epithelial cells can be noticed. Next, magnification is switched to 400x and corpuscular elements are counted in at least 10 randomly chosen visual fields. The results are recalculated per original volume of urine and reported as mean number of particles in 1 µl of urine. More accurate count of elements can be achieved by using Bürker’s chamber.

For the microscopic examination of urinary sediment a bright-field microscopy and the phase contrast technique are used; in certain specific cases also a microscopy with polarisation filter may be employed.

- **Bright-field light microscopy**
The basic light microscopy enables introductory orientation in the preparation and identification of pathologic elements. If bright-field light microscope is used for viewing a preparation without staining, some elements such as hyaline casts and bacteria can escape detection; and accurate identification of leukocytes, macrophages, and renal tubular cells is very difficult. Therefore, *supravital staining* that highlights some details of cellular structures is recommended. The term ‘supravital’ means staining of wet unfixed preparation in which some cells can still be alive. The *Sternheimer’s stain* is most recommended nowadays, utilising colored contrast of blue and red provided by Alcian Blue and Pyronine B, respectively. The Alcian Blue stains surface of cells and other elements due to a high affinity to mucopolysaccharides, while Pyronine B penetrates inside the cells and stains mostly cytosol.

- **Phase contrast microscopy**
It is a suitable technique for rapid evaluation of unstained preparations. Phase contrast enables more detailed evaluation of sediment, especially recognition of leukocytes, casts, crystals, and differentiation of red blood cells including morphologic alterations of their membranes. Better depiction of details in enabled by an increased contrast, achieved by a phase shift of the lightwave of some rays.

- **Polarisation filter microscopy**
It is suitable for better identification of crystals and lipid bodies.

**Components in urinary sediment and their evaluation**
Both organ components, represented predominantly by cells and casts, and non-organ components, such as crystals, are assessed in the urinary sediment. Also presence of microorganisms is noticed; various artifacts can occur as well. Table 7 summarizes the main components of urinary sediment.
Tab. 7 Overview of the main components of urinary sediment

<table>
<thead>
<tr>
<th>Cellular elements</th>
<th>Blood cells</th>
<th>Epithelia</th>
<th>Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>Renal tubular cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukocytes</td>
<td>Transitional epithelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>Squamous epithelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casts</td>
<td>Cell-free</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyaline</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Granular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wax</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatty</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocytic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukocytic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epithelial</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microorganisms</td>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichomonades</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mould</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystals</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Corpuscular elements in urinary sediment:

- **Leukocytes**

  Polymorphonuclear granulocytes are found most often. They appear as orbicular cells (average diameter 10 µm) with granular cytoplasm. Nucleus is segmented, but it often undergoes degenerative changes and then is difficult to distinguish from the cytoplasm. It is sometimes poorly stained; if it does stain it appears distinctly blue while the cytoplasm is red or red-brown. The appearance of granulocytes is affected by osmolality of urine. They often form clusters. The finding of granulocytes is indicative of infections in the urinary tract, if found together with erythrocytes it can be a glomerulopathy as well. In about 50% of cases bacteria are found together with leukocytes.

  A false positive finding of leukocytes can also be caused by a contamination of urine (vaginal secretion, failure to sample the middle flow of urine)

  Occurrence of lymphocytes in urine is usually associated with chronic inflammations in the kidneys, sometimes with viral infections, and also with rejection of kidney after its transplantation. A lymphocyte’s hallmark is a homogenous nucleus with a thin border of cytoplasm. The high ratio of nucleus to cytoplasm and the smooth structure of cytoplasm are the best measures for their differentiation from renal tubular cells.

  Occasionally also macrophages can be seen in the sediment. They come relatively often in urinary tract infections.

  **Physiological values:** roughly \( < 5 \) leukocytes/visual field
  \( \leq 10 \) leukocytes/1 µl urine
• **Erythrocytes**

Presence of red blood cells in urine is usually a symptom of a kidney or urinary tract disease.

Erythrocytes are smaller than leukocytes. They appear as non-nucleated discoid bodies of average diameter about 6 μm. Their size decreases with growing osmolality of urine; on the other hand at low osmolality they swell and may break down. If their hemoglobin content is low, they are difficult to recognise and appear as erythrocyte ‘ghosts’.

The appearance of urinary red blood cells can be indicative of their origin; most importantly it is possible to assess whether their source is renal (passage through glomerular membrane), or subrenal (bleeding into the urinary tract). If the glomerular membrane is severely damaged, not only proteins, but also erythrocytes can pass into the filtrate. The passage of an erythrocyte through the glomerular membrane, however, alters its shape and structure. Erythrocytes that display deviations from the ordinary discoid shape are referred to as **dysmorphic**. Some may take the shape of the ‘tyre’ (called *annular* erythrocytes); or their membranes form ‘blebs’, in this case they are called **acanthocytes**. 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. In contrast, if more than 80% of red blood cells in the sediment are **isomorphic** (normal shape), it is a non-glomerular hematuria whose source is bleeding into urinary tract due to e.g. kidney/urinary tract tumor, or urolithiasis. The hematuria originating in the urinary tract is not associated with proteinuria. For correct identification of dysmorphic erythrocytes the microscopic examination under phase contrast is required.

Causes of hematuria must always be elucidated; in particular a tumor or a severe glomerulopathy (glomerulonephritis) must be excluded.

An increased number of red blood cells in the urinary sediment can also be caused by an extreme physical labor, therapy with anticoagulant drugs or admixture of menstruation blood.

**Physiological values**: roughly < 5 erythrocytes/visual field
\[< 5 \text{ erythrocytes/1}\mu\text{l urine}\]

• **Epithelial cells**

They come from epithelial lining of renal tubuli or the urinary tract.

**Renal tubular cells**

Their occurrence in urinary sediment is **always pathological** and indicates a severe damage to the kidney, in particular **affection of the tubuli** (acute tubular necrosis, acute interstitial nephritis). The renal tubular cells are rather small (average diameter 13 μm), only slightly larger than leukocytes, of round, irregularly polygonal, cubic or faceted shape with smooth, round nuclei without nucleoli, usually not located in the cell’s centre, dark blue in stained sample. Their cytoplasm is granular, red in stained sample. Usually they come separately, but may form clusters or casts.

In unstained preparation the renal tubular cells are difficult to distinguish from transitional epithelial cells. Therefore, in clinical laboratory practice sometimes the descriptive term ‘small round epithelial cells’ is used. They can even be confused with leukocytes.

**Transitional epithelial cells**

They originate from the superficial or deeper layers of the transitional epithelium lining the urinary tract. It is not possible to determine from which particular part of he urinary tract they come.

The cells from **superficial layers** are a fairly common finding. They are **round or ovoid** with round or ovoid **nuclei localised centrally** or slightly eccentric with visible nucleoli. The cytoplasm mostly displays fine granulations (less than in tubular cells); the granulation is largely at the cell’s
periphery, rarely perinuclear. Their average diameter is about 30 μm. Finding of these cells is usually indicative of *infection in the lower urinary tract*, especially if leukocytes are also found. However, they can come even in *urine of healthy individuals*.

The cells from deeper layers are smaller (average diameter 17 μm) than the superficial ones, and much more variable in shape, which can range from ovoid to e.g. shape of club, hammer, or a cell with tails. Commonly a cell with two nuclei is found. These cells occur in urine of patients with urothelial carcinomas or urinary concrements.

**Squamous epithelial cells**

These cells are the largest ones in the urinary sediment (average diameter 55 μm), of rectangular or polygonal shape with small nuclei and abundant cytoplasm. They come from urethra or vagina and their number depends on the technique of urine sample collection. Typically, they are found in the urine from women if technique of urine collection is poor; and they have *no diagnostic significance*.

**Table 8: Morphologic features of cells found in the urinary sediment viewed under phase contrast microscope, or bright field microscope following supravital stain**

<table>
<thead>
<tr>
<th>Cellular type</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td>absent</td>
<td>discoid shape</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>segmented, lobular, bright blue,</td>
<td>granular, usually stained red</td>
</tr>
<tr>
<td></td>
<td>sometimes poorly stained</td>
<td></td>
</tr>
<tr>
<td>Macrophage</td>
<td>blue nuclei, often broken,</td>
<td>granular, usually includes erythrocytes or another phagocyted</td>
</tr>
<tr>
<td></td>
<td>non-homogenous chromatin</td>
<td>particles</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>large, smooth, filling almost the whole cell</td>
<td>thin border of cytoplasm without granulations</td>
</tr>
<tr>
<td>Squamous epithelial cell</td>
<td>degenerate, small, polygonal,</td>
<td>abundant, not particularly stained</td>
</tr>
<tr>
<td></td>
<td>localised in the middle</td>
<td></td>
</tr>
<tr>
<td>Transitional epithelium superficial</td>
<td>oval or round, usually localised in the cell centre, chromatin finely granulated, occasionally nucleolus visible</td>
<td>finely granular cytoplasm, more at cell’s periphery</td>
</tr>
<tr>
<td>Transitional epithelium deep</td>
<td>well defined, nucleoli visible</td>
<td>frequent granules, dark red, of atypical shape</td>
</tr>
<tr>
<td>Renal tubular cell</td>
<td>homogenous bright, round or oval,</td>
<td>roughly granular dense cytoplasm, often dark red, may contain lipid</td>
</tr>
<tr>
<td></td>
<td>usually not localised in the cell centre</td>
<td>inclusions</td>
</tr>
</tbody>
</table>
Fig. 1: Cells in the urinary sediment

- Erythrocytes
- Granulocytes
- Renal tubular cell
- Transitional epithelium
- Squamous epithelial cell
Fig. 2: Algorithm for identification of cellular elements in urinary sediment

- **Nucleus**
  - Absent
  - Present
- **Degenerate cell**
  - Erythrocyte
  - Round/oval
  - Lobular

- **Features of cytoplasm**
  - Only thin border
  - Abundant, vacuoles, includes phagocyted particles
  - Abundant, granular

- **Lymphocyte**
- **Macrophage**
- **Renal tubular cell**
- **Granulocyte**

- **Evaluation of cellular size**
  - Very large
  - Larger than renal tubular cell
  - Squamous epithelial cells
  - Transitional epithelium
  - Other cells
Physical analysis of urine. Urinary sediment

Tumor cells
Tumors of kidney, urinary tract and accessory organs, such as prostate, can all release tumor cells into urine. A typical such cell has markedly irregularly shaped nucleus, which is rather large compared to the cytoplasm. Unless the preparation is stained, detection of tumor cells in urinary sediment is difficult.

- **Casts** (fig. 3)
  Casts are formations of cylindrical shape, originating in distal tubuli and collection ductuli of the kidney. Matrix of the cast consists of the **Tamm-Horsfall protein** that is produced by tubular epithelial cells and protects their surface. Under certain conditions such as low pH, high osmolality, or high protein concentration, the Tamm-Horsfall protein can polymerise and produces casts of tubular lumen, which can be subsequently released into urine and seen under microscope in a urinary sediment preparation. During the cast formation, various other components can be built in – for example leukocytes, erythrocytes, renal cells, pigments (hemoglobin, bilirubin), crystals and plasmatic proteins. The casts are the only elements in the urinary sediment that for sure come from the kidney, rather than 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 cast is markedly wide. Such wide casts are hallmark of renal failure.

  The casts are classified to:
  - **cell-free**: hyaline
    granular
    wax
    fat
  - **cellular** (more than one third of cast surface consists of cells):
    - erythrocytic
    - leukocytic
    - epithelial
    - bacterial

Demonstration of cellular casts in the urinary sediment is indicative of a pathologic process in the kidney. Overview and diagnostic significance of particular types of casts can be found in table 9.

**Fig. 3: Casts in urinary sediment:**
A - hyaline cast,  C - wax cast,
B - granular cast, D – cast from renal tubular cells
<table>
<thead>
<tr>
<th>Type of cast</th>
<th>Characteristics</th>
<th>Diagnostic significance</th>
</tr>
</thead>
</table>
| **Hyaline**                  | • formed only by Tamm-Horsfall protein,  
• poor light refraction  
• not always absorb stain                                                                                                                                               | • can occur in persons without renal disease e.g. following unusual physical exercise, in fever or dehydration  
• if present in fairly high number, can indicate proteinuria                                                                                                            |
| **Granular**                 | • granules, which are visible as droplets embedded in hyaline matrix, originate from degradation of cells (tubular or blood), or proteins                                                                                                                                               | • occur in patients with proteinuria or damage of tubular cells  
• symptom of tubular necrosis (glomeruli may remain intact)                                                                                                            |
| **Wax**                      | • originate from granular casts by complete decomposition of entrapped cell debris so that inner structure is lost  
• development of a wax cast lasts several hours  
• surface is made of amorphous material  
• homogenous appearance, wide  
• sharp edges, strong light refraction                                                                                                                                 | • hallmark of renal failure or renal insufficiency  
→ ‘casts of renal failure’  
• indicates a severe proteinuria                                                                                                                                           |
| **Fat casts and casts from fatty cells** | • surface bears fat bodies made of triacylglycerol or cholesterol  
• in severe damage of glomerular membrane even the lipoproteins can pass into filtrate; they are absorbed by tubular cells that degenerate, transform into fat bodies, and end entrapped in the cast matrix                                                                                                                                 | • distinctive for glomerular damage  
• in nephrotic syndrome                                                                                                                                                    |
| **Epithelial**               | • surface of hyaline matrix bears epithelial cells peeled from renal tubuli                                                                                                                                                                                                    | • occur in patients with tubular damage                                                                                                                                  |
| **Erythrocytic**             | • erythrocytes stick on the matrix surface  
• degeneration of erythrocytes can transform them to hemoglobin casts                                                                                                                                                                                                     | • proof of hematuria of renal origin, because casts are formed only in the renal tubuli                                                                                   |
| **Leukocytic (granulocytic)** | • surface of hyaline matrix holds mostly granulocytes                                                                                                                                                                                                                       | • presence is indicative of inflammatory kidney diseases  
bacterial or non-bacterial in origin  
• proof of renal origin of leukocytes                                                                                                                                       |
| **Bacterial**                | • markedly granular, very fragile                                                                                                                                                                                                                                              | • proof of renal origin of bacteria  
• very rare, because high amount of bacteria must occur in the kidney for the cast formation                                                                            |
Urine Analysis

Bacteria
Normally, there are no bacteria in urine. Their occurrence in fresh urine constitutes a pathologic finding. Obviously, presence of bacteria in the urine can be also due to contamination during or after sample collection, as bacteria are able to proliferate rapidly during sample storage. Under microscope they appear as small coccoid or rod-like object that, unlike all other elements in the sediment, can move.

Yeast
The yeast cells are somewhat smaller than erythrocytes, oval in shape, and quite variable in size. They form clusters or chains. The yeast cells are a common finding in urinary sediment of diabetics, patients on immunosuppressive therapy, sometimes also due to administration of antibiotics.

Trichomonades
These are protozoan creatures of round or oval shape with several flagella, and rapid and jerky movements when alive. It is a common finding in urinary sediment if colpitis (inflammation of vagina) is also present.

Crystals
Examination of crystals must be performed with the early morning urine immediately after its collection. Crystals appear fairly often in the urinary sediment and their occurrence should not be overestimated. Their formation can simply result from transitory over-saturation of urine, e.g. due to intake of meal rich in urates or oxalates. Crystals form also in vitro due to cooling of the urine sample or when its pH changes. Finding of crystals under these circumstances is clinically insignificant. It is common to find crystals of uric acid or amorphous urates in acidic urine and crystals of ammonium-magnesium phosphate in alkaline urine associated with urinary tract infections.

Detection of crystals, nevertheless, has some value in patients with urolithiasis. Their presence can suggest what sort of concrements forms in the urinary tract, although finding of particular crystal type in sediment by no means constitutes proof that there is a concrement of the same chemical composition in the urinary tract. Repetitive detection of crystals is informative particularly in monitoring of patients following surgical removal of concrement, or with recurrent urolithiasis.

Identification of hexagonal crystals of cystine supports diagnosis of cystinuria. Crystals of ammonium-magnesium phosphate together with high pH of urine indicate likelihood of struvite concrements.

Profuse presence of crystalline calcium oxalate is a hallmark of intoxication with ethylene glycol; otherwise calcium oxalate crystals are common finding especially in persons with high intake of plant diet and are unrelated to formation of concrements.

Another example of informative finding is crystals of uric acid in acute urate nephropathy. Occurrence of leucine and tyrosine crystals accompanies severe liver diseases. Some drugs can also crystallise in the urine, especially in over-dosage, dehydration or hypoalbuminemia.

Table 10 and fig. 4 show features and clinical significance of selected urinary crystals.

Lipids
Lipids can penetrate into urine through damaged glomerular membrane as plasmatic lipoproteins. Since the plasma lipoprotein particles are considerably larger than plasma proteins, lipiduria is always associated with heavy proteinuria and signals severe kidney damage. Lipids can appear in the urine as free droplets, either separated or in clusters; in this case their presence can also be due to contamination from rectal suppositories. Lipid inclusions found inside the cells are a sign of cellular degeneration. Next, lipids can appear in the urine also as cholesterol crystals and fat casts.
Table 10: Features of selected crystals in urine sediment

<table>
<thead>
<tr>
<th>Sort of crystal</th>
<th>Typical shape</th>
<th>pH of urine</th>
<th>Clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acid</td>
<td>Alkaline</td>
</tr>
<tr>
<td>Urate</td>
<td>amorphous</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>variable ‘kegs’ ‘rosettes’</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>‘beads’ ‘dumbbell’</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ammonium-magnesium phosphate (triple phosphate)</td>
<td>‘cover of coffin’</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium urate</td>
<td>beads ‘spinelike apples’</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>‘envelope’ (dihydrate) ‘sponge-biscuit’ (monohydrate)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>hexagonal prisms</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>flat plates with a corner broken off</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>thin pins in bundles or rosettes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>oil beads</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4: Selected crystals in urinary sediment

![Selected crystals in urinary sediment](image-url)
Other findings

Occasionally, other elements can be spotted in the urinary sediment, such as mobile spermatozoa with long thin flagella, fibrils of mucin and fibrin, and finally various contaminating objects like textile or toilet paper fibers.

Hamburger’s count

If indicated the urinary sediment can be examined quantitatively according to Hamburger; it actually measures 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, within one hour the complete volume of collected urine is delivered to the laboratory, together with data about the time of start and end of the collection period (with precision to minutes). The laboratory examination includes counting of erythrocytes, leukocytes and casts in 5 large squares of the Bürker’s chamber.

Physiological values:

<table>
<thead>
<tr>
<th>Element</th>
<th>Up to 2000/minute, i.e., 33 Ery/s</th>
<th>Up to 4000/minute, i.e. 67 Leu/s</th>
<th>Up to 60-70/minute, i.e. 1 cast/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casts</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Automatic examination of urinary sediment

Currently, instruments for automatic examination of urinary sediment are available. They are based on the flow cytometry or digital imaging of particles.

Flow cytometry is a laboratory technique able to measure a wide array of parameters in huge number of particles. This sophisticated instrumentation has been employed mainly in hematological laboratories, but recently its usefulness also for examination of urinary sediment, so far laborious and prone to subjective error, has been increasingly recognised.

The analysed particles – cells – are labelled with various fluorophores; and the cell suspension then flows through a fine capillary. While passing the capillary, the particles encounter beam of light, usually a laser beam. The laser excites the fluorescent labels on the cells, and, in addition, the laser light is scattered by the particles. The usual measured parameters are forward scatter, which is directly proportional to the cell size, side scatter, providing information on the inner structure of analysed particles, and fluorescence of the labels employed.

The analysis of cells and other elements of the urinary sediment by means of flow cytometry can be fully automated.

Procedure

• The urine sample is mixed, aspirated into the instrument (0.8 ml), diluted and its conductivity is recorded.
• Automated staining of urinary elements with two different fluorescent dyes follows. Phenanthridine dye stains nucleic acids (orange fluorescence). The other employed stain is a carbocyanine dye that visualises negatively charged cell membranes, nuclear membranes and mitochondria (green fluorescence).
• The stained particles encounter a laser beam that is both scattered and absorbed resulting in excitation of fluorophores. Identification and counting of elements is based on evaluation of fluorescent signal from both dyes together with measurement of light scatter and electric impedance of the cell suspension.

The flow cytometer can detect all the urinary cellular elements – erythrocytes, leukocytes, bacteria and epithelial cells. In addition, it can differentiate some clinically significant entities such as isomorphic or dysmorphic erythrocytes. It reliably reports presence of pathologic casts, which however must be further examined under microscope to obtain more details. It finds also crystalline formations, but without information on their type, which again must be specified by microscopic examination. The flow cytometer cannot detect trichomonades.

Number of erythrocytes, leukocytes, bacteria, flat epithelia and casts is reported as number of elements/1 ul.

In order to increase accuracy of urinary sediment it is possible to ‘cross check’ results from flow cytometer with chemical analysis 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 way of automatic analysis of urinary sediment a sample of uncentrifuged urine is forced to fill a planar cuvette. Multiple digital images of particles present in urine are obtained with a digital camera. The size, shape and structure of imaged particles are then compared with a database in the equipment software.

Summary: Examination of urinary sediment

a) Introductory examination with diagnostic strips
   • erythrocyturia
   • leukocyturia
   • nitrites → bacteriuria

b) Microscopy: Evaluation of findings:
   • Erythrocytes isomorphic (normal)
     dysmorphic
   • Leukocytes
   • Squamous epithelium
   • Transitional epithelium
   • Renal tubular cells
   • Casts cell-free
     - hyaline
     - granular
     - wax
     - fat
   • Casts cellular
     - erythrocytic
     - leukocytic
     - bacterial

   • Bacteria
   • Crystals
   • Yeast
   • Artifacts

c) Flow cytometry or digital imaging