Compendium of urinalysis
Urine test strips and microscopy
Interesting facts
Are you aware of that …

• More than 500 million people – 10% of the world’s population – have some form of kidney damage ¹

• Urinary tract infections are the second most common type of infection in the human body ²

• One in 20 deaths is caused by diabetes; 8,700 deaths every day; six every minute ³

• By 2030, almost 23.6 million people will die from cardiovascular disease, mainly heart disease and stroke ⁴
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Main disease indications

Urine is a key health barometer for many diseases, mainly UTIs, kidney disease and diabetes. The analysis of urine can reveal serious diseases, which do not show symptoms in their early but treatable stages and causes severe damage if they remain undetected.
Urinary tract infection (UTI)

The urinary apparatus is made up of:
- Two kidneys
- Two ureters
- The urinary bladder
- The urethra

What is a UTI?
Most UTIs are caused by bacteria entering the urinary tract (urethra) and multiplying rapidly. The origin of bacteria, especially E. coli, is usually the intestines or anus.

- UTI can mean anything from a mild inflammation of the bladder to severe kidney infections, and should be treated as soon as possible
- Bacteria can spread and infect the bladder and kidneys. The only way to stop this is early detection and the prescription of antibiotics

Each day, the human body filters 170 liters of primary urine through the kidneys. Primary urine consists of water, salt and dissolved low-molecular blood constituents.
The water and all substances needed by the organism are largely reabsorbed. The remaining “worthless” substance is known as urine. The amount of urine finally excreted through the urethra each day is approximately 1.5 liters.

Fig. 1: The urinary apparatus
Main disease indications
Urinary tract infection

Symptoms and causes of UTI
Symptoms can vary, and sometimes there may be none at all. However, the most common are as follows:
• A burning sensation when urinating
• A frequent urge to urinate, but only in small amounts
• The urine may be discolored or cloudy, strong or foul-smelling
• Back or abdominal pain
• General feelings of illness or fever

Causes of UTI include:
• Sexual intercourse
• Poor hygiene
• Genetic makeup e.g. the proximity of the anus to the urethra

Risk groups
• Women are much more likely to get a UTI
• People with diabetes have a higher risk of developing a UTI due to changes in the immune system
• Any other disorder that suppresses the immune system raises the risk of a UTI

Did you know?
Research by the National Institutes of Health suggests that recurrent UTIs may be caused by the ability of bacteria to attach to cells lining the urinary tract.
A growth of E. coli bacteria in the vagina can also result from spermicidal foam on condoms.
The kidneys are the most important excretion organ in the human organism. Every 24 hours around 1,500 liters of blood flow through the kidneys.

If a UTI is not treated promptly, bacteria may travel further up the ureters to multiply and infect the kidneys.

**Function of the kidneys:**
- Filter blood to excrete water, electrolytes and metabolic end products (e.g. urea)
- Help to control blood pressure and blood pH
- Produce the important enzyme renin and glycoprotein-hormone EPO
- Help maintain healthy bones
- Excrete blood constituents (e.g. glucose)
- Maintain water and electrolyte balance
- Regulate water content of intracellular and extracellular fluid

**What does kidney disease imply?**
Kidney diseases are disorders that affect the kidneys. In many cases the tiny filtering units (nephrons) are attacked and their ability to filter properly is limited. Gradual loss of kidney function is called chronic kidney disease (CKD), and people with CKD may develop permanent kidney failure as it progresses. This may have fatal consequences unless a dialysis machine is used or a kidney transplant is performed. CKD occurs gradually and is often silent, going undetected for years. People with CKD may also have a high risk of suffering from a stroke or heart attack.

*Fig. 2: Schematic illustration of a nephron*
Nephrons – the trigger for CKD
Each kidney has between one and three million nephrons. Each nephron can be subdivided further into glomerular and tubular sections.

In the nephron, a glomerulus – a tiny blood vessel – intertwines with a tubule which collects urine. The glomerulus acts as a filtering unit and determines which substances are excreted as waste and which are reabsorbed into the blood to nourish the body’s cells, such as water, essential nutrients, salts and minerals. Afterwards, the tubules collect and concentrate waste and excrete it into the urine.

Damaged nephrons can lead to CKD and leave kidneys unable to remove wastes. The damage can occur slowly and over many years. As blood filtering becomes impaired, urine production decreases and water and waste products accumulate in the blood.

Also, substances such as protein may leak into the urine rather than remaining within the blood.

CKD can lead to kidney failure and transplantation without early detection and monitoring.

Stages of CKD
CKD develops gradually over time and can be divided into five stages of increasing severity:

Stage 1: Slight kidney damage with normal or decreased filtration
Stage 2: Mild decrease in kidney function
Stage 3: Moderate decrease in kidney function
Stage 4: Severe decrease in kidney function
Stage 5: Kidney failure requiring dialysis or transplantation to stay alive

Fig. 3: Glomerular filtration rate in correlation with CKD stages

![Figure 3: Glomerular filtration rate in correlation with CKD stages](image)
Symptoms of CKD
Unfortunately, symptoms often occur very late or not at all. Abnormal urine or blood tests are often one of the earliest signs.

Common symptoms are
• Problems urinating: burning feeling or abnormal discharge during urination, or a change in the frequency of urination, especially at night
• Urine that is foamy, bloody, red, brown
• Mid-back pain, below the ribs
• High blood pressure (hypertension)
• Lost of appetite or nausea and vomiting
• Swelling in hands, feet or eyes
• Muscle cramps

Risk groups
• People with diabetes, hypertension or obesity, and older age groups

Did you know?
Kidney disease may also occur after a bacterial infection in another part of the body, such as a streptococcus infection of the throat or skin or an infection inside the heart.7

Viruses, such as the HIV virus that leads to AIDS, can also trigger kidney disease. 1 Kidney disease requires prompt attention, and high-risk populations should be carefully monitored for abnormal kidney function.

Otherwise, testing for CKD only begins when symptoms are present, which may be too late.

Diagnostic tests, such as simple urine tests, are the first line of defense in detecting kidney problems and minimizing damage.
Main disease indications

Kidney disease
Diabetes

Diabetes, even when controlled, is the most common cause of CKD and kidney failure.

What is diabetes?
Diabetes occurs when the body fails to produce or properly utilize insulin, which is necessary for the stabilization of blood glucose levels. High blood sugar causes damage to the kidneys and other organs.

There are three types of diabetes:
• Type I (insulin-dependent or juvenile-onset): The body produces little or no insulin. It is usually genetic and is characterized by high levels of blood glucose
• Type II (insulin-independent or adult-onset): The body does not produce enough insulin, or does not use it properly (insulin resistance). It is often associated with obesity
• Type III: Gestational diabetes is hyperglycemia with onset or first recognition during pregnancy

Common consequences
Over time, diabetes can damage the heart, blood vessels, nerves and kidneys.
• The disease increases the risk of heart disease and stroke
• Diabetic neuropathy (damage to the nerves) affects up to 50% of people with diabetes
• Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure

High blood pressure and high blood glucose increase the risk that a person with diabetes will progress to kidney failure. They should therefore be screened regularly for abnormal urine protein in order to prevent the progression of CKD.

Did you know?
Healthy diet, regular physical activity, maintaining normal body weight and avoiding tobacco use can prevent or delay the onset of diabetes.
Main disease indications

Diabetes
From urine fortune telling to real-time diagnosis

It all started over 2,000 years ago. Many cultures once regarded urine as a mystical fluid, and some still do. Its uses have included wound healing, stimulation of the body’s defenses, and disease diagnosis.
The history of urinalysis

The ancient world
The origin of visual urine diagnostics, can be traced back to ancient Egypt. Hippocrates (approx. 400 BC) recognized that urine characteristics (odor / color) were altered with different diseases. He hypothesized that urine was a filtrate of the four humors (blood, phlegm, yellow bile and black bile), which came from the blood and was filtered through the kidneys and pointed out the importance of examining the patient’s urine. Six centuries later, Galen (AD 129–200) refined Hippocrates ideas, theorizing that urine represented is not a filtrate of the four humors and overall condition, but rather, a filtrate of the blood. This doctrine dominated medical thinking up to the 16th century.

The middle ages (AD 500–1500)
The technique of collecting urine was thought to be important for accurate interpretation. Ismail Gorgani, an 11th century physician, recommended collecting the full amount over 24 hours in a clean vessel and keeping it out of the sun or heat, which could alter color. The teachings of Gilles de Corbeil (1165–1213) related 20 different types of urine to conditions of the body, he noted differences in sediment and color (from crystal clear via camel hair white, blackberry red and pale green to black).

The Renaissance (1450–1600)
By the 15th century urinary diagnosis had transformed the patient–doctor dynamic. An increasing number of physicians were diagnosing from urine alone. Amateurs (called ‘leches’) started diagnosing based only on the color of urine. By the 17th century, the uses of uroscopy / uromancy had spiraled far beyond the edge of reason. Physicians and leches started telling fortunes and predicting the future with urine.
From urine fortune telling to real-time diagnosis
The history of urinalysis

**Today**
Towards the end of the 18\textsuperscript{th} century doctors became more interested in chemistry and turned their attention to a scientific basis of urinalysis. The first “test strips” were developed by the Parisian chemist Jules Maumené (1818–1898) when, in 1850, he impregnated a strip of merino wool with “tin protochloride” (stannous chloride). On application of a drop of urine and heating over a candle the strip immediately turned black if the urine contained sugar and it took another 70 years before the Viennese chemist Fritz Feigl (1891–1971) published his technique of “spot analysis.” Urine test strips in the sense used today were first made on industrial scale and offered commercially in the 1950s. The company Boehringer Mannheim, today a top leader on the world market under the name of Roche, launched its first Combur-Test\textsuperscript{®} strips (Fig. 4) in 1964.

New impregnation techniques, more stable color indicators and the steady improvement in color gradation have all contributed to the fact that the use of urine test strips has now become established in clinical and general practice as a reliable diagnostic instrument.

*Fig. 4: Roche Combur-Test\textsuperscript{®}
Applications of urine test strips

Urine test strips are a central diagnostic instrument, their ease of use yielding quick and reliable information on pathological changes in the urine. Their significance lies primarily in first-line diagnostics. Routine testing with multi-parameter strips allows a determination of the complete urine status. This is the first step in the diagnosis of a very wide range of diseases.

Indications for urine test strips:
• Screening for prevention
• Treatment monitoring
• Patient self-testing

Screening for prevention
Urine test strips are routinely used for screening both in hospitals and in general practice. The aim of screening is early identification of likely patients by examining large groups of the population.

No direct diagnoses are established on the basis of the screening results, which serve only as a basis for further microscopic, bacteriological, or clinicochemical examinations of the urine.

Routine examinations can reveal early symptoms of the following four disease groups:
• Kidney diseases
• Urinary tract infections
• Carbohydrate metabolism disorders (such as diabetes mellitus)
• Liver disease and hemolytic disorders
• Cardiovascular disease

Did you know?
A field study carried out in seven European countries with over 11,000 urine samples illustrates the value of screening with urine test strips. A pathological urine finding (after checking for nitrite, protein, glucose, ketones, urobilinogen, and blood) was diagnosed in 16% of “normal healthy persons”, in 40% of outpatients, and in 57% of hospitalized patients.

Treatment monitoring
Treatment monitoring with the aid of urine test strips allows the treating doctor to check on the results of the prescribed therapy, and if necessary to introduce any changes into the therapeutic strategy. An additional benefit of such monitoring is improved patient compliance.
Monitoring is particularly useful in two clinical conditions:
First, in diabetes mellitus, where combined checks for glucose and ketones are advisable for early detection and correction of changes in metabolic status. Second, in patients suffering from hypertension who run an increased risk of developing kidney damage in the course of their condition.

Patient self-testing
Spontaneous preventive self-testing at home has become widespread. Patients can also benefit by using test strips under their doctor’s instructions.

This applies especially to high risk groups who might develop e.g. diabetes, CKD or UTIs:
• Overweight people, and those with unhealthy lifestyles
• People with diabetes, hypertension or a family history of kidney disease
• People who have already experienced a UTI

Did you know?
Nearly 20% of women who have a UTI will have another, and 30% of those will have yet another. Of the last group, 80% will have recurrences.
Reliable analytical results can only be obtained from a urine specimen that has been collected, transported and stored properly. The first step for a correct test performance is therefore to obtain a proper sample.

**Sample collection**

Samples should always be collected in clean disposable plastic cups. Key patient data (full name, date of birth, sender, collection date and time) should be affixed to the container using waterproof materials before the sample is collected.

**Depending on the time and nature of the sample, a distinction is drawn between:**

- Spontaneous urine
- First morning urine
- Second morning urine (collected before noon)
- Timed urine (usually 24-hour urine)
- Midstream urine
- Bladder puncture urine

First morning urine has proved its worth for most test purposes. It has usually been in the bladder for a reasonably long period, and its composition is independent of daily variations in food and fluid intake and physical activity. For glucosuria tests, it is best to use urine passed about two hours after a carbohydrate-rich meal. Contamination is frequent in normal “spontaneous” urine collected without any special hygienic precautions, especially in the case of women, and consists of leukocytes in the presence of discharge and of erythrocytes in the presence of menstruation. For this reason, no urine diagnostics should be attempted in women for two to three days after menstruation.

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Fig. 5: Collecting a midstream urine sample

- Wash hands
- Take lid off specimen container and place with inside surface facing upwards
- First void a small amount of urine into the toilet, then fill the specimen container half full, void remaining urine into the toilet
- Replace the lid on the specimen container being careful not to touch the inside; give the container to the nurse or laboratory
Urinalysis can reveal diseases that have gone unnoticed because they do not necessarily produce signs or symptoms. A simple urine test can allow early detection and treatment of disease. The most efficient test strips result can be obtained after correct collection and storage of the urine sample.

**Sample storage**

Test strips should be examined within two hours of urination, since longer standing times can lead to false results owing to the following influences:
- Disintegration (lysis) of leukocytes and erythrocytes
- Proliferation of bacteria
- Bacterial degradation of glucose
- A rise in pH due to ammonia formed as a result of bacterial degradation of urea
- Oxidation of bilirubin and urobilinogen, especially in sunlight

These changes can be slowed if the urine is kept in a sealed container in a refrigerator.

There are three ways to perform a test strip analysis:
- Manual – The test is done by hand
- Semi-automated – The test strip is dipped in the urine sample manually and then analyzed by an instrument
- Fully-automated – The test strip is analyzed completely by an instrument

In the following, the focus lies on visual testing done manually. However, chapter 5 also discusses semi- and fully-automated analysis.

A general overview of storage requirements and factors influencing test results can be found in the appendix (Tab. 1).

**Use of test strips**

The test should be performed as follows:
1. Collect the specimen in a clean container and ideally decant it into a test tube to ensure even mixing
2. Dip the test strip in the urine for no longer than one second (all test pads should be fully covered in urine)
3. On drawing the strip out of the sample, run its edge over the rim of the container to remove excess liquid
4. After 60 seconds (60–120 seconds for leukocytes) compare the reaction color in the test area against the color scale on the label
5. Record the results
Macroscopic assessment of the urine is of little diagnostic value, but any striking color changes on visual examination are usually reported. The normal urine volume of an adult is 700–2,000 mL/day. An output of more than 2,500 mL/day is classified as polyuria, less than 500 mL/day as oliguria, and less than 100 mL/day as anuria.

**Color**
The color of normal urine is due to the presence of porphyrins, bilirubin, urobilin, uroerythrin, and other, still unidentified, compounds. Striking changes should be reported in terms of definite colors: “red”, “brown”, “green”, etc. Color changes are most often caused by drugs and their metabolites. A brick-red sediment is usually due to precipitation of urates in acidic urine (test: the precipitate redissolves on gentle warming). Hematuria is recognized by the presence of brown-red turbidity with a red-brown sediment.

**White turbidity can be due to:**
- Phosphates precipitating in alkaline urine (test: the precipitate redissolves on acidification with acetic acid)
- Pyuria in massive bacterial or fungal infections (microbial count >107/mL)
- Lipiduria in the presence of a nephritic syndrome or on contamination with ointments
- Massive proteinuria

**Odor**
**Striking odor changes of clinical significance include:**
- Fresh fruit or acetone in the presence of ketonuria (sign of possible presence of metabolic acidosis, most often due to fasting or uncontrolled diabetes mellitus)
- “Fetor hepaticus”, a musty odor of urine and breath in the presence of hepatic encephalopathies
- Alcohol in the presence of intoxication
- Ammonia in urinary tract infections due to urea-splitting bacteria; Hydrogen sulfide in urinary tract infections with proteinuria due to putrefacient bacteria

A general overview of color changes in the urine can be found in the appendix (Tab. 2).
Do’s
✔ Examine the test strip within two hours
✔ Mix the specimen thoroughly prior to the test
✔ If the tests cannot be done within two hours of urine collection, keep the specimen in a refrigerator at +4°C
✔ At the time of testing, ensure the samples are at room temperature
✔ Close the test strip tube immediately after removing a test strip
✔ Remember to label the urine container

Don’ts
✘ Allow contamination by residues of cleaning agents or disinfectants, as these can give false-positive findings for blood, protein and glucose
✘ Freeze the urine specimen, as this will destroy leukocytes and erythrocytes and hence make it unusable for subsequent microscopic examinations
✘ Centrifuge the specimen prior to test strip analysis
✘ Expose the specimen to direct sunlight
Characteristics of Roche urine test strips

Urinalysis is a sensitive chemical test of a urine specimen. In order to obtain an accurate result, it is important that the urine is free of contaminants which may interfere with the chemical reaction of the test pads.
Contaminants in the urine may lead to false-positive or false-negative results. Results can also be influenced by many external and internal factors, which may then lead to a missed or false diagnosis.

External factors may include contamination by preservatives or cleaning substances which enter the urine during or after sample collection.

The main internal factor which may interfere with the result is the presence of ascorbic acid.

**Nylon mesh technology**
To prevent external and internal interference, Roche has developed an unique test strip technology using a nylon mesh layer on each strip. This net sealing technology compromises a reagent and underlying absorbent paper which are fixed to a plastic carrier foil by a thin nylon mesh. The strip therefore comprises several layers hold together without glue, which might interfere with the result.

**General problems:**
- Contamination of the reagent pad by external influences and ascorbic acid
- Interferences from water-repellent glue components and possible falsification of the color by glue

**Solution:**
- Iodated components protect blood and glucose detection even with a high level of ascorbic acid (up to 750 mg/L)
- Reagent paper and underlying absorbent paper are fixed to the plastic carrier foil by a thin nylon mesh without the interventions of a glue component
- Plastic carrier foil provides extra strength and absorbent paper layers prevent splashing of urine

**Long storage life**
A drying agent in the cap of the plastic tube protects the sensitive test strips from atmospheric humidity. The test strips are stable up to the expiry date specified on the package when stored and used in accordance with the directions.

*Fig. 6: Structure of Roche test strip*
Ascorbic acid
Vitamin C (ascorbic acid) inhibits the oxidation reactions for blood and glucose in the test area and can therefore lead to false-negative results in the presence of hematuria and glucosuria.

Risks of ascorbic acid interference
An examination of over 4,000 routine urinalysis specimens provided positive results for ascorbic acid in 22.8%. The average ascorbic acid concentration is 370 mg/L, with a range of 70-3,400 mg/L. It was shown that an intake of 250 mg/day could produce a mean ascorbic acid level of 310 mg/L. The risk of false-negative results increases particularly sharply in the flu season, when some people consume large quantities of vitamin supplements, affecting the diagnosis of the following clinical pictures:

Blood: Glomerulonephritis, pyelonephritis, lithiasis, tumors
Glucose: Diabetes mellitus, glucosuria caused by kidney damage

General problems:
- Vitamin C is added to many foods and beverages due to its antioxidant and preservative properties (e.g. flour, cakes, vegetables, fruit)
- In addition, many people take prophylactically pure vitamin C in the form of vitamin tablets.

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Fig. 7: Oranges: an important source of vitamin C
**Solution:**

- Test strip protection by an iodated nylon mesh, so that ascorbic acid is eliminated by oxidation

Some urine test strips which are not protected against vitamin C offer an additional test pad for ascorbic acid instead. However, it is only possible to reveal an excessive vitamin C concentration in the patient’s urine this way. The examination should therefore be repeated at a later time to avoid potential false-negatives in the case of blood and glucose. Roche Combur-Test® strips remain stable even in the presence of high concentrations of vitamin C, and false-negative reactions to blood and glucose are hardly ever observed.

**Did you know?**

A study by Brigden et al. showed that an oral dose of as little as 100 mg of vitamin C per day, or even a single glass of fruit juice, may produce ascorbic acid concentrations of 10 mg/dL in the urine. With conventional urine test strips, these concentrations may be high enough to cause interference.¹⁰
Characteristics of Roche urine test strips

Composition and benefits of test strips
Parameters of urine strips

A urine test strip can consist of different combinations of specific reagents (chemical test pads). Each pad indicates a certain substance in the urine (Fig. 8). Special colorfast printing on the vial label allows easy and reliable evaluation of the results.

Competitive advantage
• All pads can be read 60 seconds after dipping
• The strip is readable tip-down, keeping the fingers clear of the specimen
• The strip is also readable parallel to the vial, competing products are often difficult to read because they are read perpendicular to the vial

Roche test strips satisfy all requirements for effective screening:
• The result is obtained quickly
• The test is easy and inexpensive
• High diagnostic sensitivity
• High diagnostic specificity

Benefits
• Applied nylon mesh ensures uniform color development through uniform penetration of the urine
• Separate diazonium salt mesh improves reagent stability and color differentiation in the leukocytes test
• Ascorbic acid protection avoids retesting and prevents false-negative results even with high levels of ascorbic acid
• Early detection of potential disease

Fig. 8: Roche Combur® Test® strip parameters
Characteristics of Roche urine test strips

Parameters of urine test strips
Specific gravity

Why is it important?
Specific gravity is significant in the analysis of urine for narcotics or prescribed drugs, particularly in athletes to manipulate their specimen.

Test principle
The test determines the ion concentrations in urine by reacting with a complex former and detecting the released protons.

Reference range
Values below 1,010 g/mL are of analytical significance, since erythrocytes and leukocytes undergo rapid lysis in specimens with low specific gravity. This may explain negative sediment results with a positive test strip reaction.

Diagnostic value
• Monitoring of fluid intake in patients with bladder stones
• Explains differences between microscopy and test strip results: leukocytes and erythrocytes might be lysed in low concentrated urine
• Interpretation of borderline results of test strip parameters: dilution or concentration of the urine can confirm or invalidate the pathological significance

Limitations
• The test does not indicate the contribution of non-ionic urinary constituents, such as urea, creatinine or glucose, to the specific gravity value
• In the case of urine with a pH value greater than 7.0, the specific gravity test strip reading may be too low and has therefore to be increased by 0.005 g/mL
• In the presence of protein between 100 and 500 mg/dL or ketoacidosis, the reading tends to be elevated
• An increase in urine specific gravity due to glucose concentrations >1,000 mg/dL (>56 mmol/L) is not determined

Influencing factors
The specific gravity of urine depends primarily on the amount of fluids drunk by the patient, but factors such as heavy sweating or increased urine output provoked by diuretics (e.g. coffee or certain drugs) also exert an influence, so that even in healthy persons the values can vary from 1,000 to 1,040 g/mL.
Characteristics of Roche urine test strips

Parameters of urine test strips
**pH**

**Why is it important?**
Persistently acidic or alkaline urine points to the possibility of a disturbed acid-base balance. Persistently alkaline pH values are evidence of urinary tract infection. Elevated pH values are also analytically significant because erythrocytes and leukocytes are lysed faster under these conditions, which can explain the combination of negative sediment results with a positive test strip reaction.

**Test principle**
The pH test relies on a combination of three indicators: methyl red, bromthymol blue and phenolphthalein. A pH range of 5–9 is reflected in a color gradation from orange to yellow-green and finally blue.

**Reference ranges**
Course over the day: pH 4.8–7.4
Morning urine: pH 5–6

![Fig. 9: Principle of the urine pH test](image-url)
Characteristics of Roche urine test strips
Parameters of urine strips

**Diagnostic value**

- Persistently low or high values point to the possibility of a disturbed acid-base balance
- High values occur in some bacterial urinary tract infections.
- Explanation for differences between microscopy and test strip results: lysis of leukocytes and erythrocytes at high pH values

**Limitations**

- If the specimen stands for too long, alkaline pH values are diagnostically meaningless and bacterial decomposition occurs
- Residues of disinfectants based on quaternary ammonium compounds in the sampling device may cause false results

**Influencing factors**

Nutrition such as animal protein leads to acidic urine, while a vegetarian diet may result in alkaline urine. Metabolic status and various diseases and medicines may also influence pH.

Acidosis (pH < 7) and alkalosis (pH > 7) can also be due to the following causes:

**Metabolic acidosis:**
- Diabetic acidosis
- Fasting
- Drugs and toxins
- Kidney failure
- Renal tubular acidosis (pH rarely below 6.0)

**Respiratory acidosis:**
- Retention of CO₂ (emphysema) metabolic alkalosis
- Severe potassium deficiency
- Excessive intake of alkalis
- Diuretics
- Vomiting

**Respiratory alkalosis:**
- Infections
- Fever
**Leukocytes**

**Why is it important?**
Leukocyturia is an important guide symptom for inflammatory diseases of the efferent urinary tract and kidneys, such as bacterial and abacterial infections and parasite infestations. Abacterial leukocyturia can also constitute important evidence for the presence of tuberculosis or tumors.

**Test principle**
The leukocytes excreted in the urine are almost exclusively granulocytes, whose esterase activity is detected in the test strip reaction. The test zone contains an indoxyl ester, which is cleaved by the granulocyte esterase. The free indoxyl reacts with a diazonium salt to form a violet dye.

**Reference range**
Normal < 10 leukocytes/μL  
Borderline 10–20 leukocytes/μL  
Pathological > 20 leukocytes/μL

**Practical detection limit**
10–25 leukocytes/μL

*Fig. 10: Principle of the urine leukocytes test*
Characteristics of Roche urine test strips

Parameters of urine strips

Diagnostic value

- Leukocyturia is a cardinal symptom of inflammatory diseases of the lower urinary tract, mostly caused by bacteria and the kidneys
- The leukocytes excreted in the urine are almost exclusively granulocytes, whose esterase activity is detected in the test strip reaction
- The test strip detects intact as well as lysed cells (alkaline pH > 7 or diluted urine indicated by low specific gravity), which cannot be detected under the microscope

Limitations

- The test does not react to pathogenic bacteria and trichomonads in urine
- Protein excretion in excess of 500 mg/dL and glucose excretion of over 2 g/dL could lead to weaker color development and high doses of cephalaxin and gentamicin
- Preservatives falsify the test result (false-positive reading in the case of formaldehyde, false-negative in case of boric acid). Medication with imipenem, meropenem and clavulanic acid could lead to falsepositive results

Influencing factors

False-positive leukocytes:

- Not collecting clean catch midstream, contamination by vaginal secretion or saliva
- Expired, contaminated or improperly stored strips
- Nitrofurantoin, imipenem, meropenem, clavulanic acid (antibiotics)

False-negative leukocytes:

- Specimen not mixed well or at a low temperature
- Proteinuria > 500 mg/dL
- Glucosuria > 2,000 mg/dL
- Cephalaxin, gentamycin
- Boric acid, sodium azide, mercury salts, hydrochloric acid

Did you know?

If an inflammation is chronic or has healed, it is not unusual to obtain a positive leukocyte reaction and yet fail to find any bacteria in the urine. This condition is known as “abacterial” leukocyturia. In chronic pyelonephritis leukocyturia, the only symptom is often in the intervals between the acute episodes. The additional symptoms associated with the acute course, such as fever, kidney pains, proteinuria and erythrocyturia, are absent.
**Nitrite**

**Why is it important?**
The presence of nitrite in the urine is one of the most important symptoms of a bacterial urinary tract infection. Women are particularly affected by this condition. Men suffer from these infections increasingly after the age of 60. Recognition and early treatment of urinary tract infections is of decisive importance, because a progressive infection may lead to chronic kidney failure, pyelonephritic atrophic kidneys, and uremia.

**Test principle**
The aromatic amine sulfanilamide reacts with nitrite in the presence of an acid buffer to form a diazonium compound, which is coupled with 3-hydroxy-1,2,3,4-tetrahydrobenzo-(h)-quinoline to form an azo dye. Nitrate that is present in the urine is converted by bacterial reduction into nitrite.

![Fig. 11: Principle of the urine nitrite test](image-url)
**Reference range**
Bacteria-free urine does not contain any nitrite.

**Practical detection limit**
11 μmol/L (0.05 mg/dL)

**Diagnostic value**
- The presence of nitrite in the urine is indicative of bacterial urinary tract infections (UTIs) by nitrate-deforming bacteria e.g. E. coli, independently of the pH
- On average about 50% of bacterial UTIs are detected with the nitrite test
- Under favorable conditions (first morning urine, high microbial count) more than 90% of bacterial UTIs are detected
- Screening before confirmation by bacteriological examinations
- Leukocyturia is an important supplementary finding

**Limitations**
- The intensity of the red color is a measure of the nitrite concentration but cannot be correlated to the severity of the infection

**Influencing factors**

**False-positive nitrites**
- Expired, contaminated or improperly stored strips, e.g. prolonged exposure to the air (nitrous gases)
- Drugs that color the urine red e.g. phenazopyridine
- Bacterial contamination from sample collection
- Bacteria can multiply and convert nitrate to nitrite in specimens that are more than 4 hours old

**False-negative nitrites**
- Bacteria causing UTIs may not be able to convert nitrate to nitrite
- Antibiotic therapy suppresses the enzyme metabolism and the microbial population, so that not enough nitrite is formed for the test
- Insufficient nitrate intake or too short retention of urine in the bladder

**Did you know?**
A single negative test does not exclude a urinary tract infection, because the microbial count and the nitrate content of the urine can vary. Absence of color on repeated testing is also not reliable evidence for the absence of a urinary tract infection, since a pathogenic microorganism that does not form nitrite could be present. If there is clinical suspicion of an infection, therefore, it is advisable to go on to determine the microbial species and count.
**Protein (albumin)**

**Why is it important?**
The indicator reacts particularly sensitively to albumin excreted in the presence of kidney damage. Proteinuria is a frequent symptom in renal diseases, but it is also non-specific. It is not a proof of nephropathy, nor does its absence exclude nephropathy.

**Test principle**
The detection reaction relies on the so-called protein error of pH indicators. The protein test area contains a buffer mixture and an indicator which changes color from yellow to green in the presence of protein, even though the pH is held constant.

**Reference range**
Below 10 mg/dL (for total protein)

**Practical detection limit**
6 mg/dL albumin and above

**Diagnostic value**
- The test is predominantly sensitive for albumin
- Good correlation with albumin determination by immunodiffusion
- No influence by varying pH of 5–9 and specific gravity of the urine
- No interference by quinine, quinidine, chloroquine, sulfonamides
- More convenient and generally superior to precipitation tests
- Medicines such as quinine, quinidine, chloroquine, sulfonamides and penicillin have virtually no effect on the color reaction

![Fig. 12: Principle of the urine protein test](image-url)
Limitations

- Microalbuminuria cannot be detected because the first positive result of the test strips is 15–30 mg/dL.
- The sensitivity to other proteins (e.g. γ-globulins, proteases, peptones, mucoproteins) is lower.

Influencing factors

Low or false-negative protein

- Proteinuria is mainly consisting out of other proteins than albumin.

False-positive protein

- During or after infusion of polyvinylpyrrolidone (blood substitute).
- Strongly basic urine (pH > 9) during therapy with phenazopyridine.
- Residues of disinfectants based on quaternary ammonium compounds or chlorhexidine.

Did you know?

Benign proteinuria

In persons with healthy kidneys, proteinurias are observed predominantly up to the age of 30, and account for up to 90% of the proteinurias found in this age group. The causes of these benign conditions include physical stress (for example from playing sports), emotional stress, orthostatism and lordosis. Proteinurias associated with hypothermia, heat, pregnancy, or the use of vasoconstrictively acting drugs are also usually benign. Benign proteinuria has been observed in 20% of women during pregnancy.

Renal proteinuria

An increase in the permeability of the glomerular capillaries due to pathological processes leads to the development of renal proteinuria.

In general the level is in excess of 25 mg/dL, the most pronounced proteinurias being observed in nephroses. In glomerulonephritis the protein excretion is usually 200–300 mg/dL, but lower values must be recognized within the event of glomerulonephritis associated with few symptoms. This proteinuria is usually accompanied by microhematuria.

Postrenal proteinuria

Postrenal proteinuria can occur following inflammation of the bladder or prostate and on bleeding in the urinary tract.
**Glucose**

**Why is it important?**
The determination of glucose in urine has a high diagnostic value for early detection of disorders such as diabetes mellitus.

**Test principle**
The detection of glucose is based on a specific glucose-oxidase-peroxidase reaction in which D-glucose is oxidized enzymatically by atmospheric oxygen to D-gluconolactone. The hydrogen peroxide formed oxidizes the indicator TMB under peroxidase catalysis, to give a blue-green dye which on the yellow test paper causes a color change to green.

**Reference range**
- Fasting morning urine: $<1.1 \text{ mmol/L} \quad (<20 \text{ mg/dL})$
- Daytime urine: $<1.7 \text{ mmol/L} \quad (<30 \text{ mg/dL})$

**Practical detection limit**
For ascorbic-acid-free urine the practical detection limit is around 2.2 mmol/L (40 mg/dL), so that even slightly pathological glucosurias can be detected with high reliability. The upper limit of physiological glucosuria in the first morning urine is around 0.8 mmol/L (15 mg/dL).

**Fig. 13: Principle of the urine glucose test**

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad + \quad \text{O}_2 \quad \xrightarrow{\text{GOD}} \quad \text{CH}_2\text{OH} \\
\text{OH} & \quad \text{OH} \quad \text{OH} \quad \text{H} \quad \text{OH} & \quad \text{OH} \quad \text{OH} & \quad \text{O} \quad \text{H}_2\text{O}_2 \\
\beta-\text{D-Glucose} & \quad \text{Oxygen} & \quad \text{Hydrogen peroxide} \\
\text{H}_2\text{N} & \quad \text{NH}_2 \quad + \quad \text{H}_2\text{O}_2 \quad \xrightarrow{\text{POD}} \quad \text{H}_2\text{O} \quad + \quad \text{Dye (blue)} \\
\end{align*}
\]

3,3',5,5'-Tetramethylbenzidin
Characteristics of Roche urine test strips

Parameters of urine strips

Diagnostic value
- The simplest and quickest way to screen for unidentified diabetics as well as monitoring and self-testing
- Detection of renal glucosuria, e.g. during pregnancy
- Detection of alimentary glucosuria (after extreme carbohydrate intake)
- The enzymatically catalyzed reaction sequence ensures that glucose is the only urinary constituent that will react and give a positive test result
- Ketones do not interfere, and the pH of the urine similarly does not exert any influence on the test result

Limitations
- The urine glucose concentration represents the glucose excretion during the urine collection period in the bladder, and does not necessarily correlate with the actual blood glucose value

Influencing factors
- Low or false-negative glucose
  - Metabolic products and drug metabolites which have a reducing action
- False-positive glucose
  - Presence of residues of peroxide-containing or other strongly oxidizing cleaning agents

Did you know?
The absence of glucosuria does not exclude a disturbance of the glucose metabolism, and in particular diabetes mellitus. Glucosuria develops when the tubular reabsorption of glucose in the kidneys (the renal threshold) is exceeded (Fig. 14). The renal threshold is normally at a blood glucose level of 150–180 mg/dL (8.3–10 mmol/L), but it is often elevated in older people and in persons who have had diabetes mellitus for many years.

Renal threshold for glucose

![Renal threshold for glucose](Fig. 14: Renal threshold for glucose)
Ketones

Why are they important?
Ketones (acetoacetic acid, ß-hydroxybutyric acid, and acetone) occur in the urine when increased fat degradation takes place in the organism owing to an insufficient supply of energy in the form of carbohydrates. Detection of ketones in the urine (acetoacetic acid and acetone) is particularly important in checking metabolic decompensation in diabetes mellitus.

Test principle
The detection of ketones is based on the principle of Legal’s test. Acetoacetic acid and acetone react with sodium nitroprusside and glycine in an alkaline medium to give a violet color complex. The reaction is specific for these two ketones, ß-hydroxybutyric acid does not react.

Reference range
Below 0.5 mmol/L (below 5 mg/dL) for acetoacetic acid.

Practical detection limit
The test is substantially more sensitive for acetoacetic acid (detection limit 5 mg/dL = 0.5 mmol/L) than for acetone (detection limit about 40 mg/dL = 7 mmol/L).

Diagnostic value
- Indicative of a dangerous condition for diabetic patients called ketoacidosis which can lead to coma
- Detection of starvation states
- Monitoring and detection of diet programs which severely restrict intake of carbohydrates (e.g. Atkins Diet) and zero diet
- Detection of hyperemesis gravidarum (nausea during pregnancy)

Limitations
- Phenylketone or phthaleine compounds can produce red-orange to red colors on the test pad, which are different from the violet colors produced by ketone bodies

Influencing factors
False-negative ketones
- Captopril, MESNA (2-mercapto-ethanesulfonic-acid sodium salt) and other substances containing sulphydryl

\[
\text{Na}_2 \left[\text{Fe(CN)}_5\text{NO}\right] + \text{CH}_3\text{C}–\text{R} + \text{NaOH} \rightarrow \text{Na}_3[\text{Fe(CN)}_5\text{N=CH–C–R}] + \text{H}_2\text{O}
\]

Sodium nitroprusside Ketone Color complex (violet)

Fig. 15: Principle of the urine ketones test
Characteristics of Roche urine test strips

Parameters of urine strips

Ketones
**Urobilinogen**

**Why is it important?**

Urobilinogen is excreted in increased amounts in the urine when, in the enterohepatic circulation of the bile pigments, the functional capacity of the liver is impaired or overloaded, or when the liver is bypassed.

**Test principle**

p-methoxybenzenediazonium fluoroborate, a stable diazonium salt, forms a red azo dye with urobilinogen in an acid medium.

**Reference range**

Below 17 μmol/L (below 1 mg/dL).

**Practical detection limit**

The practical detection limit is around 7 mol/L (0.4 mg/dL), at which level the urobilinogen gives normal urine a pale light pink color. Differentiation between normal and pathological urine is possible by means of color comparison. Complete absence of urobilinogen in the urine, perhaps on complete obstruction of the common bile duct, cannot be detected.

**Diagnostic value**

- Detection of acute and chronic liver diseases such as viral hepatitis, liver cirrhosis, and toxic hepatic damage
- Detection of hemolytic diseases such as hemolytic anemia, pernicious anemia, and intravascular hemolysis
- Elevated amounts of urobilinogen are indicative of compromised liver function

**Limitations**

- The test is specific for urobilinogen and does not react with other diazo-positive substances
- No red color is formed in the presence of porphobilinogen, indican, paminosalicylic acid, sulfonamides, sulfonylureas and other substances occurring in the urine

![Fig. 16: Principle of the urine urobilinogen test](image-url)
Influencing factors

False-negative urobilinogen
- Oxidation of urobilinogen if the specimen is left in direct sunlight for a long period.
- Formaldehyde > 200 mg/dL used as preservative

False-positive urobilinogen
- Drugs or metabolites which turn red in an acid medium (e.g. phenazopyridine)

Possible causes of failure of urobilinogen formation:
- Complete obstruction of the common bile duct in the absence of a bile tract infection
- Complete stoppage of bile production in the liver (very severe viral hepatitis, severe toxic liver damage)
- Absence of intestinal flora (physiological in newborn babies, rarely observed after intensive antibiotic therapy)

Did you know?
Urobilinogen is formed by bacterial reduction from bilirubin secreted into the intestine with the bile. It is then reabsorbed into the bloodstream and is subsequently broken down in the liver and partly excreted in the urine.

Urobilinogen is absent in the urine in situations involving failure of bile production in the liver cells, disturbances of bile secretion into the intestine, and absence of bilirubin reduction in the intestine, even though a severe disease may be present.
**Bilirubin**

**Why is it important?**
In all pathological processes that increase the concentration of conjugated bilirubin in plasma, the excretion of bilirubin in urine can reach considerable high levels. Conjugated bilirubin is found in the case of increased intracanalicular pressure due to an extrahepatic or intrahepatic obstruction, and with periportal inflammation or fibrosis and swelling or necrosis of the liver cells.

**Test principle**
Bilirubin reacts with a stable diazonium salt (2,6-dichlorobenzenediazonium fluoroborate) in an acid medium of the test paper. A red-violet azo dye is formed, causing a color change to violet.

**Reference range**
Adults below 3.4 mol/L (below 0.2 mg/dL).

**Practical detection limit**
The practical detection limit in urine free from ascorbic acid is 9 μmol/L (0.5 mg/dL). In favorable cases as little as 3–7 μmol/L (0.2–0.4 mg/dL) may give a positive reaction.

\[
\begin{align*}
\text{Cl} & \quad \text{N} \quad \text{N} \\
\text{Cl} & \quad \text{BF}_4^-
\end{align*}
\]

Diazonium salt

**Diagnostic value**
- Increased levels of bilirubin are found in liver disease such as icterus or obstruction of the bile flow

**Limitations**
- High ascorbic acid concentrations lower the sensitivity of the bilirubin test

**Influencing factors**

False-negative bilirubin
- Prolonged standing of the urine, particularly in direct sunlight, leads to oxidation of the bilirubin

False-positive bilirubin
- Medicines that color the urine red or that are themselves red in an acid medium, e.g. phenazopyridine
- Yellow or green reaction color of the UBG test in the presence of high bilirubin concentrations

**Fig. 17: Principle of the urine bilirubin test**
Did you know?
As a result of conjugation (esterification) with glucuronic acid, bilirubin becomes water-soluble and therefore susceptible to excretion by the renal route. The bilirubin present in urine is always conjugated (direct) bilirubin.

Diseases in which only unconjugated bilirubin is increased in the serum proceed without bilirubinuria, because unconjugated bilirubin is not excreted by the renal route. The cause may be an oversupply of bilirubin in the liver cells or a disturbance of uptake or conjugation:
- Hemolytic jaundice
- Jaundice of the newborn
- Gilbert-Meulengracht disease
- Crigler-Najjar syndrome
Blood

Why is it important?
The principal causes of hematuria, (excretion of erythrocytes in the urine) may indicate UTI, kidney disease, kidney stones or tumors.

Test principle
The test is based on the peroxidative action of hemoglobin or myoglobin which catalyzes the oxidation of the color indicator TMB by an organic hydroperoxide (2,5-dimethylhexane-2,5-dihydroperoxide) to give a blue-green dye which on the yellow test paper causes a colour change to green. High sensitivity is achieved by the addition of an activator to the reagent mixture. Intact erythrocytes are lysed on the test paper and the hemoglobin released sets off the color reaction. Visible green spots are formed. In contrast, hemoglobin dissolved in the urine (erythrocytes in lysed form) leads to the development of a uniform green color. Development of green spots (intact erythrocytes) or a green color (free hemoglobin/myoglobin) in the reagent area within 60 seconds indicates the need for further investigation.

Reference range
0–5 erythrocytes/L.

Practical detection limit
The practical detection limit for intact erythrocytes is about 5 erythrocytes/L and for hemoglobin the amount corresponding to around 10 erythrocytes/L. The practical detection limit of the test reaches the limit of the normal range.

Fig. 18: Principle of the urine blood test
**Diagnostic value**
- Detection of hematuria, a concomitant symptom of renal diseases, urinary tract disorders, and extra-renal affections
- Detection of hemoglobinuria and myoglobinuria as symptoms of hemolytic diseases, severe intoxication, extensive burns, severe muscle injuries, heavy physical strain
- Detection of intact and lysed cells
- Free hemoglobin is indicative of intravascular hemolysis
- Cellular malignancies may lead to microhematuria of unknown origin because of missing symptoms

**Limitations**
- The test is specific for hemoglobin and myoglobin. Other cellular constituents of the urine, such as epithelia, leukocytes, and spermatozoa, have no effect

**Discrepancy between test and microscopy**
- Old specimens, red blood cells (RBCs) lysed in urine upon sitting, and non-intact RBCs are not detected under microscope
- Urine not swirled, RBCs settle to the bottom, pad at the end of strip being dipped in a concentrated area
- Over-centrifugation can cause destruction of RBCs

**Influencing factors**
- False-positive blood
  - Expired, contaminated or improperly stored strips. Residues from strong oxidizing reagents in urine containers or cleansing tissues
  - Menstrual contamination, not collecting clean catch midstream

- False-negative blood
  - Formalin (used as a preservative)
  - Nitrite (in excess of 10mg/dL) delays the reaction

**Evaluation**

**Erythrocytes**
The observation of individually separated to closely set green dots on the test paper points to the presence of intact erythrocytes. At higher concentrations the dots may be so close together that the test area appears almost uniformly green. Dilution of the urine 1:10 or 1:100 with 0.9% (physiological) saline and repetition of the test with another strip will make it possible to decide whether intact erythrocytes or free hemoglobin are present.
A finding of 5–10 erythrocytes/μL requires repeated checks on the urine, and if it is obtained again must be clinically clarified.

Hemoglobin
A homogeneous green test area points to the presence of free hemoglobin, lysed erythrocytes or myoglobin. A weaker green color, as a first sign of a positive reaction, requires a repetition of the test with a fresh urine specimen. This second test may reveal, among other things, intact erythrocytes which at the time of the first test had already become hemolyzed. Persistence of the finding requires clinical clarification in all cases. In the event of a weakly positive hemoglobin reaction, the cause may also simply be heavy physical exertion. This can be easily excluded from the medical history.

Partial hemolysis
Partial hemolysis of erythrocytes present in the urine leads to the appearance on the test area of individual green dots against a diffuse green background. An exact assignment of the comparison color is then impossible, because the degree of hemolysis can be very variable as a function of age, concentration, and pH of the urine.

Did you know?
In contrast to hematuria, in which intact erythrocytes are excreted, in hemoglobinuria the urine contains free hemoglobin. This appears in the urine when erythrocytes have been broken down within the vascular system. Following intravasal hemolysis, the hemoglobin passes into the urine as soon as the haptoglobin-binding capacity of the plasma and the tubular reabsorption capacity for hemoglobin have been exceeded. This usually occurs with plasma hemoglobin concentrations of around 60 μmol/L (100 mg/dL).

Myoglobinuria is generally due to muscular injury or muscular necrosis, when the myoglobin level in plasma exceeds 9–12 μmol/L (15–20 μmg/dL).
Characteristics of Roche urine test strips

Parameters of urine strips
Detection of microalbuminuria with Micral-Test

**Microalbuminuria and its importance**
Patients with diabetes mellitus, cardiovascular disease or hypertension often suffer from a nephropathy as a late complication. Diabetes is the leading cause of kidney failure, accounting for 44% of new cases. Worldwide 50% of people with diabetes are unaware of their condition and are not treated.11

Both diabetic and hypertensive patients usually undergo a regular Micral-Test, since they are already at high risk of terminal kidney failure and to damage their cardiovascular system.

**Diagnostic value**
One factor in the early recognition of nephropathy is microalbuminuria, defined as albumin concentrations between 20 and 200 mg/L urine. Values below 20 mg/L are not critical. Early diagnosis of microalbuminuria allows an appropriate therapeutic approach in order to avoid renal failure.

The potential indications include metabolic optimization, early institution of antihypertensive therapy (preferably with ACE inhibitors), and a low-protein diet in the case of diabetics. In the case of hypertension, general measures and an effective drug therapy are indicated in order to lower blood pressure.

**Test principle**
The Micral-Test allows specific detection of human albumin in the urine by a combination of immunological and chromatographic processes. When the test strip is dipped into a sample, urine passes via a wick fleece into a layer of conjugate fleece. Then gold-labeled antibodies bind albumin and the resulting antigen-antibody complex migrates into the visualization field.

Excess antibody-gold conjugate is bound by immobilized albumin in a capture zone, meaning the detection field is reached only by conjugate molecules filled with urinary albumin. Depending on the albumin concentration, the detection field ranges in color from white to red.

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![Micral-Test](image)

Fig. 19: Micral-Test®, an Accu-Chek® product
Characteristics of Roche urine test strips
Detection of microalbuminuria with Micral-Test

Test performance

NOTE: Since the reaction of this test strip is based on chromatographic and immunological principles, pre-analytical settings slightly differ from the procedure for conventional test strips.

1. Collect first morning urine from a mid-stream, so that albumin concentration is not falsified by physical activity or fluid intake
2. Dip the test strip in the urine until the area between the two black lines is covered and hold it for five seconds. The test strip must not touch the container wall during this procedure, due to the possibility of interference effects during chromatography
3. Place the test strip on a nonabsorbent horizontal substrate or on the urine container
4. After one minute, compare the reaction color against the colors on the label
5. Repeat the test on three days in a week

Specificity and sensitivity

With a positive detection limit of 20 mg/L for microalbuminuria, the test obtains a sensitivity of 95% and a specificity of 80%. On the basis of the immunological reaction, Micral-Test measures human albumin specifically. Cross-reactions with other human proteins such as IgG, IgA, leukocytes, and erythrocytes are below 0.5%.

Evaluation

The result is considered as medically relevant if at least two of the three morning urine samples have been positive.

Limitations

Mistakes during the handling process may be due to a number of factors: the strip may be immersed too far, or for too short a time, or the reading may be taken too soon, or there may be contact between the test strip and the wet container wall.

The following findings restrict the information value of microalbuminuria:

• Acute diseases and infections of the urinary tract
• Positive urine findings for protein, nitrite, leukocytes or blood
• Pregnancy
• Severe metabolic dysregulation, for example in diabetics
• Physical exertion at the time of urine collection in the bladder (physiological albuminuria)
• Albumin of postrenal origin

Influence of drugs

Interference due to medicinal drugs has not been observed so far, but the effects of medicines and/or their metabolites on Micral-Test are not all known. If there is any doubt, and if medically acceptable, the medication should be discontinued and the test repeated.
Drug interference in urine

The results of urinalysis can be influenced by drugs, leading to false-positive or false-negative results. Roche has carried out a research study to clarify the extent of possible interference.
Influencing factors

The study is representative for all Roche urine strips and analyzing systems. It is accepted by FDA standards representing drug and chemicals susceptible to interfere with urine test strip results. Interference factors may include levodopa, sulfamethoxazol, ascorbic acid and others; a complete list can be found in the appendix (Tab. 3, 4, 5, 6). The susceptibility to interfere with the test result is determined in at least the maximum daily doses for every drug or component. If no interference has taken place the particular drug is not listed.

**Specific gravity**
No interferences were found.

**Leukocytes**

<table>
<thead>
<tr>
<th>False-positive influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin, curcumin, levodopa, nacetyl cysteine, acid or tetracycline and captopril</td>
</tr>
<tr>
<td>Lowered specific gravity</td>
</tr>
</tbody>
</table>

**Nitrite**

<table>
<thead>
<tr>
<th>False-positive influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenazopyridine from 300 mg/L</td>
</tr>
</tbody>
</table>

**False-negative influence**

| Medications with 2-mercaptoethanesulphonate-sodium (MESNA) and sulfonamide (trimethoprim, but only in extremely high concentrations (1080 mg/L)) |

The test pad is not affected by the ingestion of ascorbic acid (up to 3,000 mg/dL).

**Protein**

<table>
<thead>
<tr>
<th>False-positive influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>During therapy with p-aminosalicylic acid, chloroquine, quinidine or nitrofurantoin</td>
</tr>
</tbody>
</table>

The test pad is not affected by the ingestion of ascorbic acid (up to 4,000 mg/dL).

**Glucose**

<table>
<thead>
<tr>
<th>False-positive influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication with 2-mercaptoethanesulphonate-sodium (MESNA)</td>
</tr>
</tbody>
</table>

**False-negative influence**

| Medications with nitrofurantoin may produce false-negative readings |

The test pad is not affected by the ingestion of ascorbic acid (up to 750 mg/dL).
**Ketones**

**False-positive influence**

- Captopril, curcumin, imipenem and 2-mercaptotetanesulphonate-sodium (MESNA) or other sulphydryl-containing compounds
- Formaldehyde (stabilizer) may cause false-positive readings, but only in extremely high concentrations

The test pad is not affected by the ingestion of ascorbic acid (up to 4,000 mg/dL).

**Urobilinogen**

**False-positive influence**

- Urine from patients who are being treated with p-aminosalicyclic acid or sulfamethoxazol and phenazopyridine may show a false-positive reaction
- Urines with high pH values (pH > 9)

**False-negative influence**

- Treatments with 2-mercaptoethanesulphonate-sodium (MESNA)

The test pad is not affected by the ingestion of ascorbic acid (up to 400 mg/dL).

**Bilirubin**

**False-positive influence**

- False-positive readings are obtained during treatments with imipenem, penicillin and p-aminosalicyclic acid or due to contamination of urine container with hydrochloric acid
- Highly basic urines (pH > 9)
- Large amounts of urobilinogen in the urine affect the color change of the bilirubin test and also lead to false-positive results

**False-negative influence**

- Treatments with 2-mercaptoethanesulphonate-sodium (MESNA)

The test pad is not affected by the ingestion of ascorbic acid (up to 1,000 mg/dL).

**Blood (erythrocytes/hemoglobin)**

**False-positive influence**

- Patients with phenazopyridine medications
- High counts of leukocytes (500 LEU/μL), highly basic urines (pH > 9) and low specific gravity (< 1,005) may show false-positive test results
- Medications with 2-mercaptoethanesulphonate-sodium (MESNA) can lead to false-positive or false-negative reading of reagent pad color changes

**False-negative influence**

- When formalin is used to preserve the urine with nitrofurantoin and quinidines, false-negative results are obtained

The test pad is not affected by the ingestion of ascorbic acid (up to 1,000 mg/dL).
Automated urinalysis

The high sensitivity and specificity of Combur-Test® urine strips permit rapid and reliable conclusions about pathological changes in the urine.
It is difficult to standardize visual evaluation of urine test strips, and a number of environmental factors can have a negative effect on the quality of the result. These include:

- Differences in light conditions at the workplace
- Individual differences in color differentiation
- Declining concentration when examining long series of samples
- Differences in the accuracy of compliance with the specified test strip reaction time

Instrumental evaluation of urine test strips virtually eliminates the described factors and guarantees rapid, standardized measurement and immediate reliable documentation of the result.

**Photometry**

Urinalysis systems evaluate test strips by reflectance photometry using selective light-emitting diodes (LEDs) with a wavelength and measurement time tailored exactly to the chemical reaction and color development of the test field concerned. Compared to visual assessment, this produces improved accuracy near the limit of detection.

---

**Measuring head (sensor)**

Scheme

![Measuring head (schematic)](image)

*Fig. 20: Measuring head (schematic)*
Although urinalysis systems use reflectance photometry to evaluate the test field color changes with high precision, it is not possible to completely eliminate all differences in the composition of the sample material which could have an effect on color development. In addition, and unlike instruments for the measurement of blood glucose, urinalysis systems only yield semi-quantitative results. In calculating the result, a correction for interference by the intrinsic color of the urine is made by measuring a blank field on the test strip (compensation field), which is illustrated in the following:

- The LED (1) emits light of a defined wavelength on the surface of the test pad (2) at an optimum angle
- The light is reflected from the surface and picked up by the detector (3)
- The phototransistor sends an analog electrical signal to an A/D converter (4), which changes it to digital form
- The microprocessor (5) converts the digital reading to a reflectance value by referring it to a calibration standard
- Reflectance values are compared with the defined range limits (reflectance values that are programmed in the analyzer for each parameter)
- Outputs, semi-quantitative result (6)
- Results can be printed out or transferred to the laboratory computer

Before each measurement, the optical system is tested for variations in LED brightness and detector sensitivity. If the strip is not in the correct position, the result is not printed out and the measurement must be repeated. The result of the specific gravity test is automatically corrected if the pH value is elevated.

**Determination of measured values**

Scheme

![Reflection photometry (schematic)](image-url)
Urinalysis systems
Automated urinalysis can serve many different needs, and can be divided into three categories:

**Instruments intended for single measurements**
One test strip is manually inserted at a time. The test strip is measured automatically, and the result is delivered after about one minute. The test strip has to be removed manually afterwards.

**Semi-automatic urinalysis systems**
Test strips can be inserted manually at short intervals. Transport, measurement, and disposal of the used test strips into an in-built container are automatic. The results are automatically saved in the memory and printed out.

**Fully-automatic urinalysis systems**
Manual dipping and insertion of the test strips is not required. The urine samples are applied from sample tubes using a rotor or rack. Sample identification, test procedure and disposal of the used test strips into an in-built container are fully automatic.

Roche urinalysis systems
Roche offers a range of automated urinalysis products in all three segments, meeting a variety of day-to-day needs and based on reflectance-photometric evaluation.

The Urisys line offers standardized solutions for the ward or the physician's office as well as for low-, medium- and high-volume laboratories. The new **cobas** line enables efficient management of work and data flows in medium-volume testing sites.

The urinalysis systems can be connected to a barcode scanner for automatic sample identification. The measured results can be transferred to the laboratory computer system or PC.

**Key benefits:**
- Consistent and reliable reading
- Reliable documentation
- Standardized test procedure
- Optimized workflow

For more detailed information on product specifications, please contact your local distributor.
Automated urinalysis
Urine test strip systems

A solution that satisfies all different kind of needs

**Urisys 1100® analyzer**
Instruments intended for single measurements on wards or in physicians' offices

**Urisys 2400® analyzer**
Fully-automatic urinalysis systems for large-scale laboratories

**cobas u 411 analyzer**
Semi-automatic urinalysis systems for small to medium-sized laboratories

*Fig. 22: System overview*
Urine microscopy in differential diagnosis

Test strip urinalysis produces a high number of pathological findings requiring further diagnosis. Urine centrifugation and microscopy can be readily performed by any physician or medical technician.
Many kinds of microscopes are used in the medical laboratory. All share a basic design while differing in the special functions required by their fields of application. Their main components can be divided into devices for illumination, magnification, and contrast.

**Illumination device**
Medical specimens for microscopy are generally translucent. In transmitted light microscopy, usually known simply as light microscopy, the light source is located at the foot of the microscope.

The collector is a lens built into the foot above the light source. It collects the light and focuses it into the condenser located under the microscope stage. The amount of incident light energy is regulated using the aperture diaphragm and brightness knob. The condenser lens system is located directly under the microscope stage and uniformly illuminates the object. Using small centering screws the light field can be adjusted to the center of vision. Under adjustment the light field is enlarged using the light field diaphragm until it exactly coincides with the objective lens’ field of view. This prevents contrast-reducing light scattering (light spot too large for the objective lens) and uses the maximum amount of light energy to optimize illumination of the object.

**Magnification device**
Laboratory microscopes have an objective turret located above the object plane containing objective lenses with standard focal lengths of common magnifications (10x, 40x, 100x). The tube can contain special devices, zoom magnification systems, optical channels for multiviewer microscopy, an image camera, or a video processing system. At the observer end of the tube the eyepiece usually magnifies the image generated by the objective lens by 10x. Eyepieces may contain a corrective facility to compensate for impaired eyesight and/or reticles for taking sample measurements.
**Contrast microscopy**

The basic light microscopy method used in medicine is bright-field microscopy, for which all laboratory microscopes are equipped (Fig. 1). For urine, on the other hand, phase-contrast microscopy is the more reliable technique because highly translucent elements are less likely to be overlooked. An iris diaphragm, either separate or integrated into the condenser, forms the light to create a hollow cone, which shifts light refracted at object boundary surfaces by 90° relative to the environment when it passes through the phase ring located in a special phase contrast objective. Attenuating direct light by approximately 75% decreases brightness but considerably increases the contrast of boundary surfaces, making translucent objects more readily visible (Fig. 2). In dark-field microscopy intermediate polar filters above and below the object allow light to pass through in only one direction of oscillation. If the two filter axes are offset relative to each other, a dark field is created because light rays can only pass through the second filter if they have been deflected into the plane of the second filter by a light-refracting object. Objects are contrasted in white against a dark background (Fig. 3, 4).

*Fig. 2: Phase contrast image. Drug crystal and red blood cells with accentuated border contrast (white halo, 400x).*
Urine particles and formed elements

Urine microscopy examinations can be performed on centrifuged or non-centrifuged urine. Particles examined are cells, casts, pathogens, crystals and formed elements or insoluble compounds accumulated in the urine during the passage from the kidney to the lower urinary tract.
**Blood cells**

**White blood cells**

There are normally very few white blood cells (also known as leukocytes) in urine sediment. They only enter the urine in the presence of urinary tract inflammation. Reliable detection is therefore of particular diagnostic significance. White blood cells are recruited from blood by chemotactic signals produced by the damaged tissue. They actively migrate into the interstitium through the capillary basement membrane from where they pass through the epithelium of the renal tubules, bladder or ureter to emerge in the urine. In contrast to dysmorphic red blood cells, no changes in white cell shape have been described that betray a specifically renal origin.

**Morphology**

White blood cells are colorless, 10–12 μm in diameter, with a large nucleus, and granular cytoplasm (Fig. 5). Lymphocytes are a type of white blood cell with a large circular nucleus and a narrow peripheral cytoplasm; although they can be difficult to differentiate from macrophages in bright-field microscopy this has no diagnostic significance. Granulocytes, on the other hand, have a segmented nucleus and contain coarse granules. White blood cells are easy to differentiate from red blood cells on account of their size, nucleus, and cytoplasmic structure (Fig. 6–8). Differentiation from small tubular cells, which can also contain granular cytoplasm, may be more difficult (Fig. 7–12). White blood cells tend to adhere to each other or to other sediment constituents (Fig. 9–11).

*Fig. 5: In bright field microscopy white blood cells are approx. 10 μm in size with a prominent nucleus and granular cytoplasm (1,000x, bright field microscopy).*
Diagnostic significance
Although white blood cells in urine sediment are always a sign of urinary tract inflammation, their provenance can only be identified by studying the accompanying sediment constituents and case history. Isolated leukocyturia in which urine cultures remain negative is termed sterile leukocyturia. It suggests inflammatory disease due to pathogens that cannot be identified under the microscope and are difficult to culture. In particular it suggests chlamydial infection and renal tuberculosis. Because trichomonads are readily confused with white blood cells, a fresh urine sample should be specifically tested in such cases for motile trichomonads. In prostatitis, leukocyturia may be the only positive finding in the sediment. Sterile leukocyturia in the presence of failing renal function points to interstitial nephritis. Post-transplant lymphocyturia suggest rejection.

The presence of bacteria (see Bacteria) in addition to white blood cells in fresh urine is diagnostic of urinary tract infection in a dysuric patient. Concomitant eumorphic erythrocyturia suggests hemorrhagic cystitis or a bacterially colonized concretion (secondary renal calculus). Leukocyturia plus eumorphic erythrocyturia in the absence of bacteria suggests a toxic mucosal lesion (toxic hemorrhagic cystitis), renal tuberculosis, or interstitial nephritis. The sediment should be examined for casts. Mycobacterial cultures may be indicated. White blood cells plus dysmorphic red blood cells or acanthocytes (see Red blood cells - Morphology) suggest glomerulonephritis with concomitant interstitial nephritis or glomerulonephritis with concomitant urinary tract infection. White blood cells plus trichomonads are diagnostic of trichomoniasis. White blood cells plus crystalluria suggest crystal nephropathy.
Red blood cells
The commonest reason for investigating a patient for hematuria is a positive test strip result. If the patient is a young adult and otherwise asymptomatic, the likelihood of identifying a clinically relevant cause of bleeding is <2%. Investigati- on should only be considered after ruling out contamination, e.g. by menstrual blood. However, since hematuria can be due to very different diseases, ranging from benign hereditary hematuria and chronic glomerulonephritis to renal cell carcinoma, a rational diagnostic strategy is essential. The cause may be renal or postrenal. Invasive tests targeting the kidney, in particular biopsy, should only be performed in confirmed kidney cases. Similarly, invasive urological investigation, such as cystoscopy, is warranted only in patients with lower urinary tract disease. Evaluation of urine sediment can make a major contribution in this context.

Morphology
Not all red blood cells in urine have the normal biconcave form (Fig. 13–15). Eumorphic cells are often mixed with dysmorphic cells (Fig. 16, 17), including: acanthocytes, annular red blood cells with hemoglobin-containing membrane bulges that point either toward the center of the annulus or outward, resembling “Mickey Mouse ears” (Fig. 18); echinocytes (burr cells), with small spiny membrane projections that either run circumferentially around the biconcave annulus or spread evenly across the surface of spherical cells (Fig. 19); codocytes (target cells), stomatocytes (fish-mouth cells), and knizocytes, all of which have lost their biconcave shape and resemble spheres with one or more recesses that produce their specific dysmorphism (Fig. 20–22); schistocytes, crescent-shaped sickle cells with split edges and erythrocyte ghosts (ghost cells), empty membrane envelopes of red blood cells that have released hemoglobin into the urine through cracks in the membrane (Fig. 20, 23, 24).

Diagnostic significance
The list of potential causes of hematuria is so long that investigation has to be systematic. Initially a distinction is made between glomerular and non-glomerular causes because this considerably narrows the differential diagnostic spectrum. If the cause is not apparent from the case history or concomitant symptoms, the urine sediment must be screened for further evidence of a renal cause. Tell-tale evidence of renal hematuria consists of red blood cell casts, renal epithelia containing red blood cell casts.
Urine particles and formed elements

Blood cells

(Fig. 25, 26), red cell phages (Fig. 27), lipuduria (Fig. 28), and granular or yellow-brown casts (Fig. 29) occurring in large quantities. However, certain characteristics are only found in approximately 20% of patients. Moreover, coexistent renal and postrenal disease must be considered. Urinary tract infections, ureteric calculi, urothelial and renal cell carcinomas and drug toxicity (hemorrhagic cystitis after cyclophosphamide) also occur in lupus nephritis and chronic glomerulonephritis. If, however, no further characteristic sediment constituents are in evidence, glomerular and non-glomerular hematuria can be differentiated on the basis of erythrocyte shape. In short, urinary red cell morphology can be regarded as a reliable, simple and inexpensive diagnostic method. But how do red cell shapes arise in the urine and which shapes indicate renal bleeding? The size and shape of urinary red blood cells largely depend on three factors: urine osmolality, concomitant hemolysis, and passage through the glomerular basement membrane (GBM).

Osmolarity

If the cause of bleeding is extrarenal and the urine is iso-osmolar with blood, red blood cells in the urine are the same size and shape as those in blood vessels. In hyperosmolar (morning) urine, red blood cells shrink and assume a typical thorn-apple crystal shape. Conversely, in hypoosmolar urine (water diuresis, diuretic therapy, and diabetes insipidus), red blood cells can swell following alcohol consumption. Cell lysis giving rise to erythrocyte ghosts is increased in hyposmolar urine. Size differences due to osmotic influences have no diagnostic relevance and are not reliably recorded on microscopy (Fig. 30, 31).
Epithelial cells

**Squamous epithelial cells**
Squamous epithelial cells are a common constituent of urine sediment, in particular in women. There is often a large pellet after sedimentation, mostly made up of squamous epithelial cells. Only a small proportion stem from the urethra but their presence is evidence of mixture with vaginal secretion. Detailed instructions on the correct way to collect a midstream urine sample (swab, spread the labia etc) reduce the epithelial component considerably. In men squamous epithelium is found rarely and only in relatively small amounts.

![Vaginal squamous epithelium](image)

*Fig. 32: Vaginal squamous epithelium is characterized by large cells in a small central nucleus and a relatively homogeneous cytoplasm. Multinucleate cells are also observed (400x, phase contrast microscopy).*

**Morphology**
Squamous epithelial cells are large translucent flat cells with a small central nucleus (Fig. 32). In bright field microscopy they are often barely visible but visualization can be improved substantially by phase contrast microscopy (Fig. 33, 34). They are more or less square-shaped and their thin margins are usually folded, facilitating differentiation from other urine cells (Fig. 35). In many cases, contrasting small cytoplasmic granules are easy to pick out. These large cells can be found usually flat between the slide and the cover slip, or sometimes folded into an irregular sickle shape (Fig. 36).

**Diagnostic significance**
None in connection with renal disease. However, massive presence of squamous epithelial cells indicates contamination with vaginal secretion (Fig. 37, 38). When found in conjunction with fungi, bacteria, and white blood cells, the commonest diagnosis is vaginitis, with cystitis and other urinary tract infection being less likely (Fig. 39). If there are urethral symptoms and the midstream urine sample has been correctly collected, the most likely diagnosis is urethritis.
Renal tubular cells
The normal regeneration process means that renal tubular cells become detached from the tubular basement membrane and are eliminated in the urine in small amounts. Morphological classification is based on the fact that cells found in epithelial cell casts are different from round epithelia of the lower urinary tract. Fluorescence-labeled antibodies against Tamm-Horsfall protein have confirmed the origin of these cells based on their coating with this tubular secretion protein as opposed to transitional epithelia.\textsuperscript{15}

Morphology
Tubular cells are small round epithelial cells with a large central nucleus (Fig. 40). Differentiation from white blood cells and urothelium is not always easy (Fig. 41). Tubular cells are much smaller than transitional epithelial cells but larger than white blood cells (Fig. 42–45). They also differ from white blood cells in that they have small unlobed nuclei and a relatively homogeneous cytoplasm that contains occasional inclusions such as fat globules, red blood cells, hemoglobin, melanin, and bilirubin granules (Fig. 46). Renal tubular cells loaded with fatty particles correspond to the oval fat bodies found in the nephrotic syndrome (Fig. 47). After lengthy exposure to alkaline urine typical deformations occur, such as folding of the cell borders or condensation of the cytoplasm, with the result that cap-like attached membrane envelopes can be confused with fatty particles (Fig. 48, 49).

Diagnostic significance
In acute renal failure and other tubulointerstitial disease many tubular cells are discharged into the tubular lumen and eliminated in the urine.

Fig. 41: Tubular epithelium (right) differs from white blood cells (left and below) and red blood cells in size and cytoplasmic structure (1,000x, bright field microscopy).
Transitional epithelial cells

Urine flows from the renal parenchyma through the renal pelvis and ureter into the bladder. Desquamated urothelial cells are thus also found in the urine and must be differentiated from renal tubular cells whenever possible.

![Image: The difference between white blood cells and urothelial cells is their size (1,000x, bright field microscopy).]

**Morphology**

Urothelium comprises superficial and deep layers. Cells in the superficial layer are large and circular with a small central nucleus (Fig. 50). Measuring up to 40 μm they resemble squamous epithelial cells but the difference is that they do not have folded cytoplasmic borders. Their circular shape also differentiates them from squamous epithelium (Fig. 51). Cells in the deep urothelial layer are smaller and often have neuron-like outgrowths (Fig. 52–54). Multinuclear cells or interconnected cell aggregates occasionally occur (Fig. 55, 56).

**Diagnostic significance**

Transitional epithelial cells are also found in small numbers in the urine of healthy subjects. They can be plentiful in urinary tract infection but the number is not a reliable diagnostic criterion. In inflammatory or malignant disease of the lower urinary tract there tend to be more cells from the deep urothelial layer and large cell aggregates. If there is a clinical suspicion or otherwise unexplained eumorphic erythrocyturia, a specialist cytological opinion should be obtained.
Atypical cells
Urine microscopy is useful in the early detection of renal and lower urinary tract cancer because dysplastic mucosal cells or renal carcinoma cells advancing into the renal pelvis are discharged in the urine. Urine cytology has proven a reliable screening procedure for urothelial cancer in occupational risk groups (industrial workers exposed to aromatic nitrosamines). If clinically indicated, since cell abnormalities cannot be detected reliably by conventional bright field microscopy, targeted cytology is indicated, using cytocentrifugation and Papanicolaou staining.

Morphology
Suspect cells include multinucleated cells with prominent nucleoli and clearly vacuolated cells with an eccentric chromatin-rich nucleus. However, the exact cytological evaluation should be performed by an experienced cytopathologist.

Diagnostic significance
Conventional bright field urine microscopy is unsuitable as a general screening method for malignancy of the lower urinary tract because cell structure cannot be evaluated adequately. Also, given the low prevalence of urothelial carcinomas, many false-positive results are obtained and selective cytological tests are too labor-intensive and expensive. A chance finding of atypical cells or tumor cell aggregates is a clear indication for further cytological and urological investigation. In renal cell carcinoma the sensitivity and specificity of urine cytology are even worse. Only 27% of 436 urine samples from 31 patients with confirmed renal cell carcinoma had tumor cells in their urine.
Hyaline casts

Hyaline casts are formed in the renal tubules due to precipitation of the 95 kd Tamm-Horsfall glycoprotein, which is produced only by the tubular cells in the ascending limb of the loop of Henle and in the distal tubule and is secreted into the urine (Fig. 57). In contrast to filtered albumin that is almost completely reabsorbed in the proximal tubule, secreted Tamm-Horsfall protein accounts for the major part of physiological proteinuria. It is an important inhibitor of calcium oxalate and calcium phosphate crystallization in the urine. It is highly likely that Tamm-Horsfall protein is of pathophysiological significance as an antigen in chronic tubulointerstitial renal disease. Precipitation of Tamm-Horsfall protein fibrils to form hyaline casts is elevated in low tubular flow (dehydration by fever, sport), presence of X-ray contrast agents, Bence-Jones proteinuria (myeloma cast nephropathy), and acid urine, whereas they dissolve quickly in alkaline urine (e.g. due to bacteria). This explains why hyaline casts can also be detected in urine specimens of healthy persons.

Morphology

Hyaline casts are sharply delineated, colorless, and translucent. As a result, even relatively large amounts can sometimes be missed in bright field microscopy (Fig. 58). In phase contrast microscopy, however, they are easily recognized even by an inexperienced observer due to their birefringent border delineation (Fig. 59). If it is not possible to perform phase contrast microscopy, hyaline casts become more readily visible if the condenser below the stage is moved to its uppermost position (Fig. 60). Since the light beam is smaller, the cast borders are less overexposed and the depth of focus is improved. Hyaline casts can be compact, fibrous, or coiled, due to the varying density of the protein fiber network (Fig. 61–63). Their width is a function of the inner diameter of the tubular lumen in which they formed. Wide casts are common in chronic renal insufficiency because the damaged nephrons have a dilated lumen due to tubular cell atrophy. Often the tip of the cast contains granular inclusions that turn into a pure hyaline cast and thus represent a transitional form of granular casts (Fig. 64). The situation is the same with isolated cellular inclusions which, like pure red blood cell casts or white blood cell casts, constitute evidence of a renal cell origin (Fig. 65–68). These must be differentiated from cellular urine constituents that have only become attached to hyaline casts extrarenally or during centrifugation (Fig. 69). However, by adjusting the micrometer screw it is usually possible to differentiate attached cells from cells enclosed in the cast.
Diagnostic significance

The detection of pure hyaline casts is of no significance in the diagnosis of renal diseases. They are a consequence of a changed tubular environment, as occurs physiologically in healthy subjects and pathophysiologically after extrarenal changes in hemodynamics and hydration status.\(^{25, 26}\) Physiological Tamm-Horsfall proteinuria becomes visible by precipitation in the form of hyaline casts. Renal diseases, however, involve precipitation-inducing factors, with the result that hyaline casts can be detected to an increased extent in glomerular and tubulointerstitial diseases.\(^{27}\) Beyond diagnostically significant microscopy findings (white blood cell casts, acanthocyturia), evidence of hyaline casts does not provide further useful clinical information. It should be noted that in a renal manifestation of light chain disease (multiple myeloma kidney, myeloma cast nephropathy) only hyaline-like casts are occasionally found in urine sediment, consisting of a precipitate mixture of light chains and Tamm-Horsfall protein. Morphologically they cannot be reliably differentiated from hyaline casts. If cellular inclusions are detected in the hyaline cast matrix, diagnostic significance is higher if these cells can be shown to be of renal origin. However, a hyaline cast with a single embedded red blood cell does not provide the same diagnostic certainty of glomerular disease as the detection of multiple red blood cell casts. Physiologically, red blood cells migrating through the glomerular basement membrane can be embedded in hyaline casts when passing through the nephron.\(^{28}\) Such findings should prompt close inspection of several samples because in renal disease either a number of similar casts or distinct red blood cell casts can be expected. Diagnostic certainty increases with the number of cellular inclusions. In short, hyaline casts are commonly encountered formed elements that are only significant in the diagnosis of renal disease if there is evidence of cellular inclusions.
Granular casts
As with hyaline casts, the matrix of granular casts consists of the tubular secretion of Tamm-Horsfall protein. If there are relatively small cell constituents, cell degradation products, or protein precipitates in the tubular lumen during precipitation of the glycoprotein, they are fixed in the forming matrix and create a cast of the corresponding tubular segment. Inclusions of granular casts in the urine examined under a microscope can have various origins. In glomerular or proximal tubular damage they are precipitates of glomerular filtered serum proteins or degradation products of renal cells that are already fractionated by the loop of Henle when they enter the distal tubule, where together with Tamm-Horsfall precipitates they form granular casts (Fig. 70, 71). In tubulointerstitial disease they are disintegrated tubular cell constituents that are eliminated in the form of granular casts (Fig. 72, 73). However, since further degradation of cellular products also takes place in the cast matrix, even intact, enclosed tubular cells or white blood cells take the form of granular casts in time (Fig. 74). Cell borders or nuclei are then no longer apparent. During passage through the nephron granular casts can be transformed into waxy casts. Electron microscopy, immunohistochemistry, and immunofluorescence with antibodies against serum proteins and tubular cell antigens have all been used to establish the varying composition of granular casts, enabling differential diagnosis between acute glomerular disease and tubular necrosis, for example. However, these methods are not used in routine diagnosis.

Morphology
Granular casts are sharply delineated and contain finely to coarsely granular inclusions, the cell borders of which cannot be visualized reliably (Fig. 75–77). They are readily visible even at low levels of magnification (10 x 10) (Fig. 78). The surface is rougher than in hyaline casts because they mostly form in damaged tubules (Fig. 79). If the contrast is particularly high, pigmenturia etc. must be considered (see Yellow-brown casts) (Fig. 80). The casts can be differentiated from hyaline casts covered by a bacterial lawn on the basis not only of the high number of bacteria but also their intrinsic motility at higher magnification (also at 10 x 100 under oil immersion microscopy) (Fig. 81). Amorphous crystals can also cover hyaline casts or, when clustered in groups as pseudocasts, can be confused with granular casts (Fig. 82). This can be observed at low magnification as patchy sediment typical of amorphous crystals and, with the 40 x objective, by the absence of sharp cast delineation.

Fig. 77: Granular casts are easy to recognize in bright field microscopy (400x, bright field microscopy).
Diagnostic significance
A certain amount of tubular cell degradation is physiological. Whether the degradation products appear in the form of granular casts mainly depends on the precipitation of Tamm-Horsfall protein. Consequently, a small number of granular casts can be found in the urine of healthy persons. That also explains the granular inclusions in the matrix of hyaline casts. However, an increased discharge of granular casts is found in many glomerular and tubulointerstitial diseases (Fig. 83).18 In erythrocyturia or leukocyturia, without confirmed cast evidence, the increased discharge of granular casts can be a strong sign of renal disease (Fig. 84). In addition, granular casts are also often found in acute renal failure due to tubular necrosis, rhabdomyolysis, or toxic renal damage (Fig. 85).

Pigmented casts
The renal discharge of pigmented serum constituents such as hemoglobin, myoglobin, and bilirubin can lead to the formation of yellow-brown casts. The relatively large amounts of hemoglobin released in hemolysis are transported by haptoglobin to the liver where conversion to bilirubin allows clearance via the bile. If intravascular hemolysis is substantial, however, the transport capacity of haptoglobin is exceeded with the result that hemoglobin is freely filtered in the kidney. In hemochromatosis, or two to three days after intravascular hemolysis, hemosiderin granules can also be detected in the urine. Myoglobin released following skeletal muscle damage (molecular weight approx. 40,000 daltons) is always filtered and thus found in the urine.30 Rising serum creatinine values and a positive urine dipstick test for blood are an indication for urine microscopy which will show massive amounts of yellow-brown myoglobin casts instead of intact red blood cells. In obstructive jaundice, filtered conjugated bilirubin stains the sediment constituents yellow. Consequently, granular casts that were otherwise previously colorless can appear yellow.

Fig. 90: Stained granular casts can also be reliably differentiated from confounders (400x, bright field microscopy)

Morphology
Even low magnification shows a patchy brown „soiled“ sediment with a large number of yellow-brown granular casts, some of which are short (Fig. 86). Higher magnification shows medium to coarse-grain granules with a yellow-brownish color embedded in a hyaline matrix (Fig. 87–90). Since myoglobinuria can lead to acute renal failure, epithelial cell casts can be a common sign of tubular necrosis.
**Diagnostic significance**

Cast color alone is not a reliable guide to the type of pigment. A urine test strip can help in classification based on the results of hemoglobin/erythrocyte and urobilinogen test pads. Patients with bilirubinuria are always jaundiced. Hemoglobinuria can be differentiated from myoglobinuria by examining the patient serum. Free hemoglobin stains the serum reddish.

**Waxy casts**

Waxy casts are rarer than hyaline casts and never found in subjects with healthy kidneys. Their width suggests that they form in ectatic nephrons with minimal flow.

Fig. 91: Waxy casts are wide homogeneous casts with a sharp border and rounded corners (1,000x, bright field microscopy).

**Morphology**

Waxy casts are wide and translucent with a high refractive index, which is why they are more visible than hyaline casts under bright field microscopy. Their borders are sharply delineated and the corners perpendicular (Fig. 91). Lateral "slits" are an indication of the compressed spiral structure, as is occasionally well visualized in an unfolded form (Fig. 92–94). It is assumed that proximal cast structures formed in non-ectatic tubular segments fold up in a corkscrew-like manner in distal ectatic tubules. Sometimes this spiral structure is particularly evident due to inclusions of relatively small granules in the nucleus of the waxy cast (Fig. 95, 96). In rare cases fine granular casts are also surrounded by a „waxy coat“ and thus represent a mixed form with granular casts (Fig. 97).

**Diagnostic significance**

Wide waxy casts are only found in advanced renal diseases with considerable morphological tubulointerstitial changes (Fig. 98). Occasionally, waxy casts can be detected in acute or chronic rejection of renal transplants as well as in the polyuric phase following anuric renal failure.
Red blood cell casts

Red blood cell casts form due to fixation of red blood cells located in the tubular lumen in the precipitated matrix of Tamm-Horsfall protein or occasionally without a protein matrix due to pure compression of a large number of red blood cells in the tubules (Fig. 99).\textsuperscript{21,22,23} They are of high diagnostic significance in clarifying unexplained hematuria. However, even if the cause of renal bleeding is identified, they can often only be found in small numbers. If clinically warranted, systematic inspection of the entire specimen, and possible further specimens, is advisable. For this purpose the edge of the cover glass should first be examined for suspicious casts with the 10x objective, which can then be observed in detail with the 40x objective. Sensitivity can be increased by prefiltering large amounts of urine.\textsuperscript{31}

![Fig. 99: Red blood cell casts developing in a Tamm-Horsfall matrix in renal tubules (hematoxylin-eosin, 1,000x, courtesy Dr. Weis, Pathological Institute, University of Munich).](image)

Morphology

Red blood cell casts can consist of a hyaline cast with the inclusion of single red blood cells or numerous red blood cells in a hyaline matrix (Fig. 100–102). Although dysmorphic red blood cells are regarded as being typical of glomerular bleeding, the red blood cells enclosed in casts are usually eumorphic (Fig. 103). They can be differentiated from other cell casts or granular casts by their yellowish color, the partially retained double border structure of the red blood cells, and the absence of nuclei or nucleolus-like structures. However, in interstitial renal disease, mixed-cell casts are often found with the inclusion of red blood cells, white blood cells, and renal tubular cells (Fig. 104–108). In the case of pure red blood cell casts, the formed elements are only several millimeters long (Fig. 101). If they stay in the nephron for any length of time, red blood cells enclosed in casts can degenerate to the stage at which their cell borders can no longer be recognized (Fig. 109). Emerging hemoglobin stains the cast matrix in such a way that it is no longer possible to differentiate them from yellow-brown casts occurring in rhabdomyolysis or hemolysis. However, clinical symptoms and the often concomitant hematuria with acanthocyturia generally facilitate differentiation.

Diagnostic significance

Red blood cell casts are evidence of a renal origin to hematuria because red blood cells can only pass from the bloodstream to the tubular lumen through the glomerular or tubular basal membrane. However, the sensitivity of red blood cell casts as a marker of renal hematuria is only just above 50%.\textsuperscript{35,36} Glomerulonephritis and systemic inflammatory disease involving the kidney are the commonest cause of red blood cell casts, especially in the presence of concomitant proteinuria. The number of casts provides information about the inflammatory...
activity of the disease. If proteinuria is absent or only minimal, this suggests IgA nephritis and the thin basement membrane syndrome (benign familial hematuria). Isolated proteinuria is characteristic of diabetic nephropathy and further sediment findings tend to suggest an additional renal disease. Nevertheless, in patients with diabetic nephropathy and glomerular hematuria with red blood cell casts a different glomerulopathy is only found by histological analysis in some cases. Traumatic renal damage and embolic renal infarctions are further causes of glomerular hematuria with the formation of red blood cell casts. Temporary discharge of red blood cell casts can be found in subjects with healthy kidneys following heavy physical exercise such as marathon running. In renal tuberculosis all types of sediment constituents can be found. In addition to the characteristic findings of sterile leukocyturia, glomerular hematuria with casts can also occur occasionally.

**White blood cell casts**

In inflammatory renal diseases, infiltrating white blood cells are released into the tubular lumen. They can either be enclosed in a matrix of precipitated Tamm-Horsfall protein or can conglomerate directly to form a matrix-free cast. Although the white blood cells have to overcome the tubular basal membrane, dysmorphic forms do not occur as with glomerular red blood cells. This is due to the ability of white blood cells to lyse cell to cell contacts and basal membranes in diapedesis and actively migrate through them.

**Morphology**

Isolated white blood cells can be enclosed in hyaline casts. However, crowded clumps of white blood cells within or without a hyaline matrix are regarded as genuine white blood cell casts. Temporary discharge of red blood cell casts can be found in subjects with healthy kidneys following heavy physical exercise such as marathon running. In renal tuberculosis all types of sediment constituents can be found. In addition to the characteristic findings of sterile leukocyturia, glomerular hematuria with casts can also occur occasionally.

**Diagnostic significance**

White blood cell casts represent evidence of inflammatory infiltration of the renal interstitium, as in pyelonephritis, interstitial nephritis, renal tuberculosis, transplant rejection, and interstitial coinvolvement in glomerulonephritis.

**Epithelial cell casts**

If tubular basement membrane sheds several tubular cells into the lumen, epithelial cell casts form due to mechanical compression or embedding in the hyaline matrix of precipitated Tamm-Horsfall protein.
Morphology

Epithelial cells originating from a tubular segment often accumulate as a typical double row of roundish cells to form an epithelial cell cast, whereas white blood cell casts consist of a disorganized, compressed amount of small cells (Fig. 116). But epithelial cell casts can also occur in a disorganized manner if the tubular cells originate from different tubular segments (Fig. 117–119). Nevertheless, unstained they can usually be differentiated from white blood cell casts by their smaller cell size, often polymorphic nuclei, and absent nucleoli (Fig. 120). Only in isolated cases can a Giemsa stain be performed to differentiate lymphocytic white blood cell casts from epithelial cell casts or to produce reliable evidence of mixed cell casts. Often only single epithelial cells are enclosed in an otherwise hyaline cast (Fig. 121–123).

Diagnostic significance

Epithelial cell casts are found in interstitial renal disease. They confirm the renal origin of other isolated round epithelia.

Fatty casts

Serum lipids bound to lipoproteins are not normally filtered by the glomerular membrane. Albumin-bound or free fatty acids that pass through the glomerular basement membrane are completely reabsorbed in the proximal tubule and are thus not normal constituents of urine. However, in renal and extrarenal disease with a disorder of the filtration barrier they tend to enter the tubular lumen and can be enclosed in hyaline casts when passing through the nephron.

Morphology

Fat globules can be enclosed singly in hyaline casts in the form of small translucent spheres or they can fill them completely (fatty casts) (Fig. 124). Lipid globules are round, translucent, and of variable size. Fatty particles consisting of pure cholesterol esters are smaller, have a higher refractive index, and shine brightly in polarized light with a characteristic Maltese cross (Fig. 125, 126).
Diagnostic significance
Fatty casts as a sign of lipiduria are a typical sediment finding in patients with substantial proteinuria or nephrotic syndrome. Apart from glomerulonephritis, fatty casts can also occur in non-glomerular renal disease, extrarenal disease, and lipid storage diseases.33, 34, 35

Cylindroids
The shape of cylindroids would appear to be somewhere between casts and mucosal strands. However, since casts do not form in the reproductive organs, the thin portion of cylindroids has nothing to do with mucus. On the contrary, it is assumed that it at least partially corresponds to the thin lumen of the loop of Henle.

Morphology
In sediment they are characterized by a narrow, longish, spindle-like structure that occasionally can be confused with hyaline or waxy casts (Fig. 127). They also have a sharply delineated border but at one end they lack the blunt end typical of casts, which is drawn out like a tail instead. Confusion with mucosal strands is especially possible under bright field microscopy if the formed hyaline portion of the cylindroid is overlooked. In phase contrast microscopy, however, differentiation is easy. Deposits of granules, red blood cells, white blood cells, and fatty particles are comparable with pure casts (Fig. 128–132).

Diagnostic significance
Fogazzi reports that cylindroids in 85 of 90 sediments were associated with cell-containing casts. In this respect cylindroids are comparable with casts in terms of diagnostic significance.21

Rare casts
Bacterial casts
Easily overlooked in bright field microscopy, bacteria-containing hyaline casts can rarely be found in phase contrast microscopy. It is easy to confuse them with hyaline casts coated by bacterial lawn (Fig. 133, 134). Genuine bacterial casts are almost always a sign of bacterial pyelonephritis.22, 23, 24 Certainty can only be obtained by considering the case history and evaluating a urine sample immediately after collection.

Yeast casts
Yeast deposits in hyaline casts are found in Candida sepsis with renal involvement.47 They are easily confused with granular or red blood cell casts (Fig. 135, 136).

Platelet casts
Casts of agglomerated platelets with disseminated intravascular coagulation are extremely rare in sepsis.
Crystal casts
Crystals often form a cast shape (Fig. 137, 138). It is difficult to distinguish whether these structures originated in the kidney or only formed in the bladder. Concomitant hematuria suggests a renal origin, which is confirmed if small crystals are enclosed in a large hyaline cast (Fig. 139, 140). However, crystal deposits can look deceptively similar (Fig. 141). At all events the evaluation can only be performed in conjunction with the patient’s case history and clinical picture.

Pseudo casts
If the urine sediment contains primarily crystals, they are usually densely packed aggregates that can occasionally be mistaken for granular casts (Fig. 142). Also in erythrocyturia or leukocyturia aggregates caused by centrifugation can give rise to cast-like structures (Fig. 143, 144). Certain artifacts can also resemble casts (Fig. 145–148).

Fig. 142: This pseudo cast is formed by calcium phosphate crystals (1,000x, polarized light).

Morphology
Pseudocasts due to aggregations are usually short. Even by adjusting the micrometer screw it is not possible to detect a sharp cast border. Due to the blurred outline of these structures, they may sometimes be confused with hyaline casts with inclusions of bacteria, cells, or crystals.

Diagnostic significance
None.
Pathogens

Trichomonads
Trichomonas vaginalis is a unicellular pathogen responsible for anorectal and urogenital infections. It is transmitted by sexual intercourse or contact infection.

![Trichomonads](image)

*Fig. 149: Trichomonads are either round or oval and can easily be confused with white blood cells or epithelial cells (1,000x, bright field microscopy).*

Morphology
Trichomonas vaginalis has a central nucleus and at approx. 15 μm it is 1.5 to 2 times larger than a white blood cell. Thus in terms of size it can tend to be confused with small round epithelia (Fig. 149). Its axial rod, which projects beyond the cranial pole, is rarely distinct. The 3 to 5 flagella of the caudal pole are constantly in motion, with the result that in fresh urine microscopy trichomonads can easily be differentiated by their motility from white blood cells and epithelial cells. A lateral membrane emerging from the flagellar pole also serves motility and is the reason for the often blurred border contour at one end (Fig. 150). By adjusting the micrometer screw it can be seen as a wavy line slightly above or below the plane in which the border contour can be sharply visualized.

Diagnostic significance
Trichomonads can cause vaginitis or urethritis, or merely represent fecal contamination. Trichomonas hominis is an apathogenic bowel commensal, which is why the isolated finding of trichomonads in asymptomatic patients can be meaningless. In symptomatic trichomoni- sasis white blood cells are found in large numbers, making it easy to overlook trichomonads (Fig. 151). Consequently, in a patient with urethral symptoms and isolated leukocyturia, trichomonads should be sought in a freshly processed urine sample as the cause of trichomonas urethritis. If there is evidence of contamination with vaginal secretion (squam- ous epithelial cells, mucosal strands), this would tend to suggest trichomonas vaginitis.
Urine particles and formed elements
Pathogens

**Fungi**
The fungi frequently found in urine are usually Candida. More accurate differentiation is not usually required because all fungi are sensitive to common antimycotics.

![Image: Yeasts and red blood cells](image)

*Fig. 152: At low magnification yeasts cannot be differentiated from red blood cells (100x, bright field microscopy).*

**Morphology**
Yeast such as Candida albicans are 5–7 μm in size, colorless, and have round to oval bodies that can easily be confused with red blood cells or certain uric acid crystals (Fig. 152, 153). Thanks to the double forms or short chains occurring during budding differentiation can nevertheless be reliable, although there can be a certain similarity with protrusions of acanthocytes (Fig. 154). However, if 5% acetic acid is added, fungi fail to dissolve, as opposed to red blood cells. Vegetative fungal forms (hyphae) are almost always found in addition, or tubular chains of different lengths subdivided by short septa (Fig. 155–157). Entire networks of hyphae with roundish blastocytic sprouting form a mycelium (Fig. 158, 159). Other forms of yeasts are found rarely (Fig. 160).

**Diagnostic significance**
Fungal spores are found physiologically on the skin and in the air. Pure contamination of midstream urine is possible but rare. Candida albicans is a frequent pathogen of vaginitis: squamous epithelia and mucosal strands in the urine sediment are clear evidence that the fungi are of vaginal origin. Contamination-free urine collection is essential. If there is no such indication in women or if fungi are found in the urine of men, white blood cells in the urine and a positive test strip result for glucose can confirm a urinary tract infection with Candida in a diabetic, usually following instrumental intervention. In rare cases spores in the urine of severely immunosuppressed patients are evidence of systemic Candida infection, having been filtered and eliminated in the urine (Fig. 161). Fungal casts forming in this way are a rarity but they represent evidence of fungemia.
Bacteria

Although urine is normally sterile, bacteria can very often be detected in urine sediment.

Morphology

95% of all bacteria responsible for urinary tract infections are Gram-negative rods (E. coli, Proteus mirabilis, Klebsiella pneumoniae), present singularly or in short chains, usually in large numbers (Fig. 162, 163). In hospitalized patients Gram-positive cocci (enterococci) are of significance. Among these, staphylococci can be differentiated from amorphous urates or phosphates on account of their characteristic intrinsic motility (Fig. 164). Bacteria are much smaller than red blood cells and even after lengthy examination they are still carried through the microscopic field by small currents. Their tendency toward adherence can lead to confusion with formed sediment constituents because epithelial cells, hyaline casts, and white blood cells can be completely covered by a bacterial lawn (Fig. 165).

Diagnostic significance

Two questions are relevant in the detection of bacteria:

1. Is bacteriuria present? If bacteria are found in urine sediment, bacteriuria is not necessarily present. Contamination by vaginal secretions, from the collection vessel, or due to bacterial growth resulting from lengthy storage are important factors that make it difficult to interpret the bacterial evidence as bacteriuria. With a doubling rate of as short as 20 minutes for the most frequently detected microorganism in urine, Escherichia coli, it is therefore only understandable that bacteriuria can only be confirmed if the urine has been collected correctly and processed immediately. Presence of squamous epithelial cells suggests contamination with vaginal secretions. In the event of clinical relevance it is therefore necessary to carry out an immediate urine processing or select a more reliable method of urine collection (midstream method/bladder puncture).
2. Is a urinary tract infection present? Urinary tract infection is the most frequent bacterial infection in humans. Three factors are of significance in its diagnosis:

a) Pyuria is a crucial sediment finding for a urinary tract infection (Fig. 166). If squamous epithelial cells are also found, vaginitis must also be considered. However, if bacteriuria is significant, in only 50% of patients is it possible to detect more than 5 white blood cells per microscopic field by conventional urine microscopy (10 x 40, high-power field). In the event of appropriate clinical suspicion a white blood cell count must be performed in a Rosenthal chamber. With this technique 96% of symptomatic patients have more than 10 white blood cells/mm³, as opposed to less than 1% of asymptomatic patients.

b) If symptoms are typical, bacteria in the sediment are an important sign. However, asymptomatic urinary tract infections must also be reliably diagnosed and treated, e.g. in the case of diabetics or neurological cases.

c) Due to inevitable bacterial contamination in the collection of urine the term significant bacteriuria was created. At least for Gram-negative microorganisms, colony-forming units of more than 10⁵ determined in dip culture plate tests are significant. Mixed cultures suggest contamination. Nitrite strip tests are an indirect sign of significant bacteriuria. Together with a positive white blood cell stick test the predicted value for a urinary tract infection is 97%. Streptococci and staphylococci are not typical pathogens of urinary tract infections. Their detection in the urine either suggests contamination or is a very serious sign of septic spread, e.g. endocarditis with filtration of circulating bacteria into the urine. The latter is highly likely in the case of fever accompanied by pyuria and an alarming diagnosis (Fig. 167). Genuine bacterial casts are only to be found in ascending urinary tract infections (Fig. 168).
Crystals

Crystals in urine sediment are particularly fascinating for the examiner because they occur with a large diversity of shapes and colors. Even if only a few are diagnostically relevant, one feels the compulsion to classify the crystals. Since a single chemical compound can occasionally assume different crystal forms depending on temperature, pH, and other factors or form various crystals similar to substances, reliable differentiation is sometimes only possible with knowledge of urine pH and polarization behavior. Since commercial urine test strips permit approximate orientation using pH, correct determination is usually possible without having to conduct further solubility tests. Apart from drug crystals the diagnostically relevant crystals are only found in slightly acidic urine (leucine, tyrosine, cystine, cholesterol). Crystal behavior in polarized light is valuable. Most crystals appear in different colors when exposed to singly polarized light (birefringence). The various forms of uric acid crystals are especially easy to confirm this way. In the case of calcium oxalate the diamond-shaped dihydrates differ from polymorphic monohydrates on account of different behavior in polarized light as the latter appear in all colors. Leucine and tyrosine spheres can be differentiated as only leucine crystals exhibit a color reaction. In alkaline urine only calcium phosphates remain colorless in polarized light.

**Calcium oxalate**

Oxalic acid is taken in with food and is an intermediate metabolic product. Dissolved calcium oxalate crystallizes out at a urine pH that is normal or slightly acid to slightly alkaline so the crystals are a frequent concomitant finding of otherwise unremarkable urine samples. Unlike red blood cells they are insoluble in 5% acetic acid but dissolve in hydrochloric acid.

**Morphology**

Calcium oxalate crystals in a regular octahedral form are monohydrates (Fig. 169). They are clear and translucent so when the micrometer screw is adjusted, first the front edges and then the rear edges can be visualized in the focal plane. The respective edge outside the focal plane shows through as a black or white line so calcium oxalates were described as having the shape of an envelope. However, due to their strong light refraction they are more like sparkling diamonds. Calcium oxalate crystals are usually smaller or approximately the same size as red blood cells but they can also be of gigantic size.
Crystals

Main disease indications

Crystals

size (100 μm) (Fig. 170). Occasionally, round, oval, or hourglass-shaped calcium oxalate crystals are also seen (Fig. 171–178). At low magnification round forms can be confused with red blood cells (Fig. 179).

**Diagnostic significance**

None, because oxalate and calcium are physiological constituents of urine. If they are repeatedly found in fresh urine in large amounts, quantitative tests for hyperoxaluria must be performed.

**Uric acid**

Uric acid is a soluble metabolite of urine metabolism. A high purine diet, congenital enzyme defects, and rapid cell degradation can all cause large amounts of uric acid to be secreted in the tubules and eliminated in the urine. If the solubility product is exceeded, crystals can precipitate in the kidney where concretions can form. The solubility of uric acid depends on the pH. If urine pH is acid, physiological uric acid concentrations precipitate as crystals. Vice versa, they can be dissolved immediately by alkali. After calcium oxalates they are the next most frequently found urine crystals.

![Fig. 180: In bright field microscopy one can recognize uric acid crystals on account of their highly refringent properties. The forms, on the other hand, can vary considerably (1,000x, bright field microscopy).](image)

**Morphology**

Uric acid crystals occur in a wide variety of shapes and sizes. They are clear and transparent to dense brown in color. Most frequently they have the shape of rhombic plates or whetstones that rarely exceed the size of red blood cells (Fig. 180). When agglomerated, they form rosettes or cover cellular sediment constituents. Contaminants such as hairs or fibers serve as seed crystals, which can be covered by a whole layer of crystals. Barrel-shaped, rosette-shaped, rod-shaped, or hexagonal uric acid crystals are seen in rare cases. They polarize in interference light (Fig. 181–186).

**Diagnostic significance**

None because uric acid is a physiological constituent of urine. They merely confirm an acidic urine pH. However, large aggregates, if detected repeatedly, can suggest primary or secondary hyperuricosuria.
Amorphous urate
If urine pH is neutral or slightly acid, uric acid is physiologically present as a urate due to the detachment of a proton.

Morphology
In urine, amorphous urates do not take the form of needles as in a joint biopsy specimen but that of small dark grains. Clusters easily form which, even in an overview, give the entire sediment a soiled appearance (Fig. 187). Urates in urine cover all other sediment constituents, which gives hyaline casts a granular exterior (Fig. 188). Nevertheless, urates that have precipitated in tubules in urate nephropathy can occlude the tubular lumen as urate casts (Fig. 189). In terms of form, amorphous urates are no different from phosphates. When exposed to polarized light, urates show a colored contrast.

Diagnostic significance
Apart from urate nephropathy, in which large amounts of urate casts are discharged, the detection of amorphous urates is of no diagnostic value.

Amorphous phosphate
If urine pH is slightly alkaline, amorphous phosphates are often detected.

Morphology
In bright field microscopy they cannot be differentiated from amorphous urates (Fig. 190). However, they do not form a contrast in polarized light.

Diagnostic significance
None. Amorphous phosphate is present in alkaline urine and can only be identified as such without chemical analysis if the pH is known.
**Calcium phosphate**

In urine, calcium phosphate is normally found dissolved in solution but it can also precipitate to form crystals in an alkaline environment.

![Image: Highly refringent calcium phosphate crystal (400x, phase contrast microscopy).

**Morphology**

The highly birefringent crystals are colorless and can take on very different forms (Fig. 191, 192). They usually form scaly crystals that can agglomerate to form entire groups (Fig. 193). Grayish granular plates or small prisms also occur (Fig. 194, 195).

**Diagnostic significance**

None.

**Magnesium ammonium phosphate**

Ammonium magnesium phosphate crystallizes out in alkaline to neutral urine. It dissolves when acetic acid or hydrochloric acid is added.

![Image: The symmetry and colors of magnesium ammonium phosphates are particularly attractive (400x, polarized light).

**Morphology**

The colorless crystals are highly refringent so they can shine in all colors when exposed to interference light (Fig. 198). Due to the typical prismatic shape they are also termed coffin lid crystals or envelope crystals (Fig. 199–202). Small to very large crystals can occur alongside each other.

**Diagnostic significance**

Like amorphous phosphate, magnesium ammonium phosphate is a sign of alkaline urine pH.
Rare crystals

**Dicalcium phosphate**
Dicalcium phosphate crystals can occasionally be seen in alkaline or slightly acid urine. They dissolve when acetic acid is added.

![Fig. 204: Dicalcium phosphate and amorphous phosphate (400x, bright field microscopy).](image)

**Morphology**
Dicalcium phosphates are colorless crystals that are druse or fan-shaped and take the form of single wedge-shaped rods (Fig. 203, 204).

**Diagnostic significance**
None.

**Dimagnesium phosphate**
In alkaline urine dimagnesium phosphates sometimes precipitate and dissolve again in acetic acid.

![Fig. 205: Alkaline urine occasionally contains large, translucent dimagnesium phosphate plates (400x, bright field microscopy).](image)

**Morphology**
The colorless flat rhombic plates have sharp irregular edges and occur in various sizes that look like shattered pieces of broken glass (Fig. 205–209). They are highly refringent but remain colorless in polarized light.

**Diagnostic significance**
None.
Tyrosine
Tyrosine crystals only precipitate in acid urine. They do not dissolve in ethanol or acetic acid.

Morphology
Tyrosine precipitates in the form of long needles. Usually it is typical rosettes that form, which can easily be confused with other crystals (Fig. 210).

Diagnostic significance
Tyrosine crystals are occasionally detected in patients with hepatic insufficiency.

Leucine
Leucine crystals also only precipitate in acid urine and dissolve in hot acetic acid.

Morphology
The morphology of leucine crystals is characteristic. Leucine has small circular crystals with concentric rings (Fig. 211–213). In polarized light it appears colored.

Diagnostic significance
Like tyrosine crystals leucine spheres are only found in patients with advanced hepatic insufficiency.

Hippuric acid
These rare crystals are found after increased consumption of food containing benzoic acid.

Morphology
Various forms such as needles, prisms, or rhombi can occur.

Diagnostic significance
None.
**Cystine**

Even if considerable amounts of cystine are eliminated in the urine, cystine crystals are only found in acidified urine from pH 4.

Fig. 214: Cystine crystals usually occur as flat hexagonal plates that are colorless (courtesy Dr. E. Wandel, Nephrology, University of Mainz, 400x, phase contrast microscopy).

**Morphology**

Cystine crystals have a regular hexagonal shape. They are flat and colorless (Fig. 214). In rare cases the crystals cluster to form rosettes (Fig. 215).

**Diagnostic significance**

In patients with calculus-induced ureteral obstruction the urine sediment should be acidified, after routine examination, with acetic acid to establish to a pH <4 to search for cystine crystals. The crystals are diagnostic of cystinuria, a rare genetic defect causing urolithiasis in homozygous individuals.

**Ammonium biurate**

Ammonium biurate forms after the splitting of urea by urease-forming bacteria. Crystals are only found at an alkaline urine pH.

Fig. 216: Ammonium biurate spheres can scarcely be confused (400x, bright field microscopy).

**Morphology**

Ammonium biurate crystals are clearly identified by their dark brown color and round shape (Fig. 216).

**Diagnostic significance**

None. Even in urinary tract infections ammonium biurate spheres are rarer than ammonium magnesium phosphate crystals.
Main disease indications

Rare crystals
Crystal formation from drug metabolites is heavily dependent on solubility, the administered dose, and urine pH. Initial reports go back to the use of early sulfonamides (acetylsulfadiazine, acetyl sulfamethoxazole), which are hardly used nowadays. Nevertheless, drug crystals can even now be found originating from a number of common medicinal products. Due to the use of high doses of cotrimoxazole for the treatment of opportunistic infections in the presence of AIDS, both crystalluria and complications (hematuria and acute renal failure) have been reported (Fig. 217, 218).\textsuperscript{54, 55}

With other antibiotics as well, such as fluoroquinolone, amoxicillin, but also aciclovir, dose-dependent crystalluria occurs particularly at an acid urine pH (Fig. 219).\textsuperscript{32, 56, 57} In the case of antiretroviral protease inhibitors that are used for the treatment of HIV infection drug crystalluria is an important side effect (Fig. 220–222).\textsuperscript{58} Unilateral or bilateral urinary tract obstructions or even acute renal failure have been described. On account of these substances symptomatic crystalluria, which became rarer after the introduction of newer sulfonamides, has returned to the differential diagnosis of hematuria and acute urinary tract obstructions.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{indinavir_crystals.png}
\caption{When exposed to polarized light indinavir crystals shine in all colors (400x, polarized light).}
\end{figure}
Main disease indications

Drug crystals
Other constituents

Fat globules and cholesterol crystals
Apart from fatty casts lipiduria in urine sediment can be recognized by individual fat globules, oval fat bodies, lipid granules in epithelial cells, and cholesterol crystals.

Morphology
Lipids can be present in urine in the form of globules or crystals. Lipid globules are round, translucent, and of various sizes (Fig. 223). Fused into small clusters they are described as oval fat bodies (Fig. 224, 225). Tubular cells or macrophages can contain relatively large numbers of lipid granules absorbed from the lumen by endocytosis (Fig. 226). If they consist of pure cholesterol esters, they shine brightly with a characteristic Maltese cross in polarized light. Cholesterol crystals are colorless rhombic plates with angled recesses that result in cube formations when lying on top of each other (Fig. 227, 228). They are only noticed in phase contrast microscopy. Fat globules and cholesterol crystals are instantly soluble in ether but not in acid nor alkali. Sudan stains lipid granules and globules deep red.

Diagnostic significance
See fatty casts.

Spermatozoa
Spermatozoa or sperm cells can occasionally be found in both male and female urine samples.

Morphology
On account of the arrow tip-like to oval head and the long tail sperms cannot be confused even by an inexperienced investigator (Fig. 229). However, they are only sufficiently visible at a high magnification (400x). In maturation disorders (following chemotherapy) various types can occur (Fig. 230). In a fresh urine sample it is occasionally possible to observe the intrinsic motility of the spermatozoa.

Diagnostic significance
In women, spermatozoa suggest contamination with vaginal secretion.
Contamination
Urine microscopy aims to help recognize diseases of the kidneys and lower urinary tract. For this purpose it is important that artifacts and urine sample contaminations are detected reliably in order to prevent confusion with genuine urine constituents. Contaminations can enter the urine sample from patients themselves (skin, feces, clothing) and during processing in the laboratory.

Vaginal secretion
The most frequent urine contamination originates from vaginal secretion. Relatively large amount of mucus can be found in bright field microscopy and additionally provide good contrast in phase contrast microscopy (Fig. 231, 232). Other solid sediment constituents easily adhere to the long fibrous mucosal strands (Fig. 233). Usually squamous epithelial cells are also usually found in large numbers. Spermatozoa in women also suggest contamination with vaginal secretion. If white blood cells and yeasts or bacteria are found, an increased level of vaginal secretion due to vaginitis must be considered. In diagnostically relevant cases women must be briefed about how to perform the midstream method properly before another urine sample is examined under the microscope (Fig. 234).

Skin
Desquamated skin scale and hairs can be found in centrifuged urine. Hairs can easily be differentiated from other structures on account of their shape, smooth surface, and split ends (Fig. 235, 236). Skin care products are also found in urine in the form of fat globules or talcum particles (Fig. 237–239). When examined by dark field microscopy, talcum particles manifest a distorted Maltese cross, as opposed to fat globules (Fig. 240).

Feces
Lack of physical hygiene or fistulas can lead to contamination of the urine sample with fecal matter. Under the microscope this is only noticed when constituents that are normally only found in feces are found in sediment. This particularly applies to bacterial clusters, meat fibers, or worm eggs eliminated enterally. (Fig. 241)

Clothing
Material fibers can enter the urine sample directly from clothing of the patient, of the assisting nursing staff or laboratory assistant, or through the air. They are characterized by their length and highly refringent properties. White blood cells can become attached to the fibers secondarily and cause confusion with casts (Fig. 242). In many cases, fibers are covered with crystals (Fig. 243). This proves that many of the crystals found in urine only crystallize out after removal in the sample vessel.
Sample vessel
Even disposable vessels can already contain fibers, dust, pollen, or production residues before the urine sample is taken, which are discovered by urine microscopy following centrifugation.

Air
Contamination of urine with matter suspended in the air occurs in every laboratory. Especially in spring and summer the dispersion of pollen leads to regular contamination with flower pollen, which can occasionally cause uncertainty (Fig. 244). Some rare crystals such as ammonium urates can easily be confused with pollen particles (Fig. 245). However, contamination is never restricted to a single sample. Air bubbles enter the urine following centrifugation when the sediment is agitated rigorously, or when the cover glass has not been applied properly (Fig. 246). They are clearly evident due to sharp delineation. They do not refract light so they can be clearly differentiated from lipid globules in dark field microscopy.

Latex gloves
Talcum or latex particles, which can easily be confused with amorphous crystals or fat globules, enter the urine from laboratory gloves (Fig. 247, 248). In polarized light or dark field microscopy these particles are represented by a distorted Maltese cross, which allows distinct differentiation from fat globules (Fig. 249, 250).

Glass splinters
Occasionally, tiny glass splinters, which resemble certain crystals, enter the urine from glass materials being used such as cover glasses or centrifuge tubes. However, differentiation is usually possible due to the irregular shape of the splinters. Also it is not possible to dissolve them by acidification or alkalinization.
Main disease indications

Other constituents
Diagnostics

A microscopy examinations yield important and detailed information in the detection and evaluation of renal and urinary tract disorders, infections as well as other systemic diseases.
Renal diseases

Glomerulonephritis
In glomerulonephritis or inflammatory systemic disease involving the kidney, “active nephritic sediment” is regarded as the detection of glomerular hematuria with acanthocyturia and red blood cell casts, as well as more or less severe proteinuria (Fig. 251, 252). White blood cells, hyaline casts, and granular casts are usually also present. However, urine sediment analysis does not result in characteristics which would differentiate the type of glomerulonephritis.

Acute pyelonephritis
If signs of a lower urinary tract infection with dysuria, frequency, bacteriuria, and leukocyturia are accompanied by flank pains, fever, or shaking chills, an ascending infection with involvement of the renal pelvis is likely. In terms of histopathology the medulla is infiltrated with neutrophilic granulocytes. The glomeruli are not affected. The urine sediment analysis corresponds to symptomatic infection of the lower urinary tract with involvement of the renal pelvis is likely. In terms of histopathology the medulla is infiltrated with neutrophilic granulocytes. The glomeruli are not affected. The urine sediment analysis corresponds to symptomatic infection of the lower urinary tract with severe leukocyturia (Fig. 253). Usually a high number of renal epithelia are also found as a sign of tubular structure involvement. Although anatomically the disease is interstitial nephritis, granular, epithelial and white blood cell casts are only seen rarely because the renal sediment proportion is low due to concomitant infection of the lower urinary tract (Fig. 254).

Endocarditis
In endocarditis the kidneys can be affected by various complications, some of which can be differentiated by urine sediment analysis. The most obvious complication is embolism of bacteria-containing valvular deposits in renal arteries. In urine sediment eumorphic hematuria and bacteriuria are in evidence, and possibly leukocyturia as well. Cell-containing casts and renal epithelia are not typical but they can be found. If staphylococci or streptococci are found, diseases such as osteomyelitis, dental root abscess, catheter infection, or introduction of other foreign matter must be considered as the source of septic focal nephritis. Dysmorphic red blood cells, red blood cell casts, and proteinuria tend to suggest concomitant glomerulonephritis, as occurs not only in rheumatic fever but also in other types of endocarditis in the form of peri-infectious (endocapillary proliferative) glomerulonephritis. Occasionally, changes in renal function, leukocyturia with white blood cell casts, granular casts, eosinophiluria, and few eumorphic red blood cells are only found in the course of and after the commencement of antibiotic treatment. In such cases acute interstitial nephritis must be suspected as an allergic or toxic side effect of any of the antibiotics used.
**Nephrotic syndrome**

The nephrotic syndrome is clinically characterized by severe proteinuria (>3.5 g/24 h), hypercholesterolemia, hypoalbuminemia, and edema. The cause is always a disorder of the glomerular filter. A large number of serum proteins enter the primary urine and exceed the capacity of the proximal tubular cells for reabsorption. Purely interstitial renal diseases or diseases of the lower urinary tract cannot be the cause of a nephrotic syndrome. Diagnosis of a nephrotic syndrome is made clinically and on the basis of appropriate laboratory parameters. Quantification of proteinuria is performed by the chemical test methods. Proteinuria is not directly evident in microscopy. Hyaline casts are precipitates of physiological Tamm-Horsfall protein and not of serum proteins. Only by the discharge of cholesterol particles or the tubular cells damaged secondarily by proteinuria is it possible to infer severe proteinuria from sediment (Fig. 255, 256). Tubular cells that have become detached from the tubular basement membrane due to maximum reabsorption of proteins are discharged in urine as vacuolated round epithelial cells. In the case of substantial hyperlipidemia fatty particles also accumulate in tubular cells which, when shed into the tubular lumen, appear in the sediment as oval fat bodies. If cellular portions are enclosed in the lumen in a Tamm-Horsfall matrix, they appear in the urine as fatty casts (Fig. 257, 258). However, only chemically determined protein detection in the urine is used in monitoring.

**Acute tubular necrosis**

Renal failure due to acute tubulointerstitial damage leads to necrotic shedding of the tubular epithelium into the tubular lumen. There are many different causes of acute tubular necrosis. At most, urine microscopy can help to support any existing suspicion. Many small round epithelial cells are always observed. The cytoplasm is often densely filled with vacuoles. Tubular cell casts and granular casts are characteristic of the simultaneous shedding of entire tubular cell aggregates in (Fig. 259–262). Eumorphic red blood cells, red blood cell casts, and white blood cell casts are often found in addition (Fig. 263). If myoglobinuria or hemoglobinuria exists as the cause of acute renal failure, large numbers of yellow-brown casts are formed (Fig. 264). Differentiation is performed with the aid of serum parameters.

*Fig. 260: This sediment contains granular casts, amorphous phosphates, and drug crystals as a sign of toxic renal failure (400x, phase contrast microscopy).*
**Bacterial cystitis**

In a typical case, bacterial cystitis can be reliably diagnosed with a urine strip test and sediment microscopy, without further diagnostic measures. White blood cells and bacteria are found in fresh midstream urine. As concomitant findings urothelia and red blood cells (hemorrhagic cystitis) can be observed occasionally (Fig. 265–267). Even though white blood cells are often found on a massive scale, the diagnosis is no less likely at lower white cell counts. On the other hand, a count of less than 10 white blood cells/mm$^3$ in the Rosenthal chamber is insufficient to diagnose a urinary tract infection. Significant bacteriuria is supported by a positive nitrite test strip result. Squamous epithelial cells, hairs and foreign matter in the urine, however, suggest contamination.

**Vaginitis/urethritis/balanitis**

If white blood cells and pathogens are found in the urine, it does not necessarily indicate a case of bladder inflammation. Bacterial growth in the urine, urethral inflammation, or an infection of the vagina can also be the cause (Fig. 268). Since vaginitis, for example, would be adequately treated by local therapy (vaginal suppositories), differentiation is also of clinical significance. Pyelonephritis, cystitis, urethritis, and vaginitis can naturally be sufficiently distinguished by careful study of case history. However, squamous epithelial cells, mucosal strands and hairs suggest contamination with vaginal secretion (Fig. 269). Fungi are a very rare cause of cystitis and nephritis but they account for the most frequent pathogen group in vaginitis (Fig. 270, 271). If white blood cells and squamous epithelial cells are found in the urine of men with dysuria, it is most likely to be a case of urethritis or balanitis. If no bacteria are found in the urine, arrangements should be made for a chlamydia smear test.
Diagnostics

Urogenital diseases
Appendix
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## Storage conditions, influencing factors and interference factors

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<th>Influencing factors</th>
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<tr>
<td>Specific gravity</td>
<td>4–8° C 20–25° C</td>
<td>Fluid intake, diuretics</td>
<td>pH &gt; 7</td>
<td>Precipitation changes the specific gravity</td>
</tr>
<tr>
<td>pH</td>
<td>Unstable Unstable</td>
<td>Diet (meat ↓, vegetarian ↑)</td>
<td>Strong color of urine ↑</td>
<td>Rise on formation of ammonia</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>1–4 h 1–4 h</td>
<td>Vaginal secretion</td>
<td>High glucose and protein values ↓</td>
<td>Fast lysis at specific gravity &lt; 1.010 and pH &gt; 7</td>
</tr>
<tr>
<td>Nitrite</td>
<td>8 h 4 h</td>
<td>Bacterial count</td>
<td>Strong color of urine ↑</td>
<td>Mix urine specimen well</td>
</tr>
<tr>
<td>Protein (albumin)</td>
<td>7 days 1 day</td>
<td>Physical activity, pregnancy</td>
<td>Ejaculate ↑</td>
<td>Antibiotics inhibit nitrite formation</td>
</tr>
<tr>
<td>Glucose</td>
<td>8 h 2 h</td>
<td>Pregnancy, fever, old age</td>
<td>Preservatives ↑</td>
<td>Test is more sensitive to acetoacetic acid than to acetone</td>
</tr>
<tr>
<td>Ketones</td>
<td>6 h 2 h</td>
<td>Starvation, fasting, fever</td>
<td>Phenyketones ↑</td>
<td>Oxidation in air</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td></td>
<td></td>
<td>Phthaleins ↑</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
<td></td>
<td>SH compounds ↑</td>
<td></td>
</tr>
<tr>
<td>Blood (erythrocytes)</td>
<td>1–4 h 1–4 h</td>
<td>Menstruation, strong physical activity</td>
<td>Light ↓</td>
<td>Oxidation in air</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strong color of urine ↑</td>
<td>Fast lysis at specific gravity &lt; 1.010 and pH &gt; 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phenazopyridine ↑</td>
<td>Mix urine specimen well</td>
</tr>
</tbody>
</table>

*Tab. 1: Storage conditions, influencing and interference factors*
Tab. 1 (Continued): Storage conditions, influencing and interference factors

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Stability in urine</th>
<th>Influencing factors</th>
<th>Interference factors</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>In sediment:</td>
<td>4–8°C 20–25°C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacteria</td>
<td>24 h 1–4 h Unstable</td>
<td>Urinary pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>1–4 h 1–4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>1–4 h 1–4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>1–4 h 1–4 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low pH, antibiotics, infections outside the bladder (kidney stones, prostate), fastidious microorganisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indwelling catheter, collection technique (children, old persons), delayed working-up</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cells are lysed in dependence on pH and osmolality. Osmolality < 300 mmol/L reduces storage stability.

Results too low or false-negative results.

Results too high or false-positive results.
<table>
<thead>
<tr>
<th>Color / appearance</th>
<th>Endogenous causes</th>
<th>Suspicion of</th>
<th>Exogenous causes Drug</th>
<th>Foods</th>
<th>Intoxications / infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorless</td>
<td>Polyuria</td>
<td>Diabetes mellitus</td>
<td>Quinine</td>
<td></td>
<td></td>
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<tr>
<td>Yellow</td>
<td>Bilirubin</td>
<td>Bilirubinaemia</td>
<td>Phenolphthalein</td>
<td>Anthrone (rhubarb)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methyldopa</td>
<td>Carotene Vitamin B2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrofurantoin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown-red</td>
<td>Haemoglobin</td>
<td>Haemoglobinuria</td>
<td>Phenytoin</td>
<td>Sulfamethoxazol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>Myoglobinuria</td>
<td>Deferoxamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phenazopyridine (orange)</td>
<td>Betanin (beetroots)</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>Porphobilin</td>
<td>Porphyria</td>
<td>Deferoxamine</td>
<td>Rhodamine B (orange)</td>
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</tr>
<tr>
<td></td>
<td>(darkening)</td>
<td></td>
<td>Phenytoin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>Bile</td>
<td></td>
<td>Amitriptyline</td>
<td>Pseudomonas</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Evans blue</td>
<td>Resorcinol</td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td></td>
<td></td>
<td>Methylen blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>Haemoglobin</td>
<td>Massive haemolysis</td>
<td>Levodopa (darkening)</td>
<td>Phenols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(darkening)</td>
<td>in malaria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melanin</td>
<td>Melanoma</td>
<td>Metronidazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homogentisate</td>
<td>Alkaptonuria</td>
<td></td>
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</tbody>
</table>
Appendix

Color changes in urine
## Drug interference factors

<table>
<thead>
<tr>
<th>Interference factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>N-Acetyl-Cysteine</td>
</tr>
<tr>
<td>Salicyluric acid</td>
</tr>
<tr>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Calcium dobesilate</td>
</tr>
<tr>
<td>Cefoxitin</td>
</tr>
<tr>
<td>Gentamycine sulfate</td>
</tr>
<tr>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Levodopa</td>
</tr>
<tr>
<td>Methyldopa</td>
</tr>
<tr>
<td>Phenazopyridine</td>
</tr>
<tr>
<td>Ofloxacine</td>
</tr>
<tr>
<td>Tetracycline</td>
</tr>
</tbody>
</table>

*Tab. 3: Interference factors*
### Tab. 4: Interference factors from package inserts of Combur¶Test®M/UX / Chemstrip® 10 UA / Chemstrip 10 A

<table>
<thead>
<tr>
<th>Interference factor</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td>Gentamycin sulfate</td>
<td></td>
</tr>
<tr>
<td>Phenazopyridine</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td></td>
</tr>
<tr>
<td>Imipenem = Primaxin*</td>
<td>Imipen + Cilastin</td>
</tr>
<tr>
<td>Meropenem = Meronem*</td>
<td>Meropenem</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td></td>
</tr>
<tr>
<td>Polyvinylpyrrolidone</td>
<td></td>
</tr>
<tr>
<td>Phenylketone</td>
<td></td>
</tr>
<tr>
<td>Phthalein compounds</td>
<td></td>
</tr>
<tr>
<td>2-Mercaptoethanesulfonate-sodium (MESNA)</td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td></td>
</tr>
<tr>
<td>Quarternary ammonium groups</td>
<td>Mecetronium etilsulfat</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td></td>
</tr>
<tr>
<td>Oxidizing cleaning agent</td>
<td>Hypochlorite</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>Trichomonads</td>
<td></td>
</tr>
<tr>
<td>Epithelia cells</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Ketones</td>
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</tr>
<tr>
<td>Urobilinogen</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>Specific gravity</td>
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*Tab. 4: Interference factors from package inserts of Combur¶Test®M/UX / Chemstrip® 10 UA / Chemstrip 10 A*
<table>
<thead>
<tr>
<th>Interference factor</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulhydryl-containing compounds</td>
<td>N-Acetyl-Cysteine</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>Trimethoprim</td>
</tr>
<tr>
<td>Captopril</td>
<td></td>
</tr>
<tr>
<td>Quinine</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>Glybenclamide</td>
</tr>
<tr>
<td>p-Aminosalicyc acid</td>
<td></td>
</tr>
<tr>
<td>Indican</td>
<td></td>
</tr>
<tr>
<td>Porphobilinogen</td>
<td></td>
</tr>
<tr>
<td>Boric acid</td>
<td></td>
</tr>
<tr>
<td>Spermatozoa</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td></td>
</tr>
<tr>
<td>Stercobilinogen</td>
<td></td>
</tr>
</tbody>
</table>

*Tab. 5: Interference factors from the Compendium*

<table>
<thead>
<tr>
<th>Interference factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazol</td>
</tr>
<tr>
<td>Curcumin</td>
</tr>
<tr>
<td>Trimethoprim</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>i-Propanol (70%)</td>
</tr>
</tbody>
</table>

*Tab. 6: Additional interference factors*
Appendix

Drug interference factors
Figures

Fig. 1: Bright field image. Drug crystal with substantial central summation effects (400x).

Fig. 2: Phase contrast image. Drug crystal and red blood cells with accentuated border contrast (white halo, 400x).

Fig. 3: Dark field image. The yellow drug crystal as in Fig. 3 viewed in dark field microscopy. Strong contrast with fewer superimposition artifacts (400x).

Fig. 4: Dark field microscopy. Visibility in dark field microscopy describes the refringent properties of an object, a fatty particle in this case.

Fig. 5: In bright field microscopy white blood cells are approx. 10 μm in size with a prominent nucleus and granular cytoplasm (1,000x, bright field microscopy).

Fig. 6: At low magnification white blood cells are seen on a massive scale in a patient with bacterial cystitis (100x, bright field microscopy).
Microscopic urine sediment

Figures

Fig. 7: At higher magnification there are not only granular white blood cells but also relatively small red blood cells representing a sign of hemorrhagic cystitis. Below: three round epithelia (400x, bright field microscopy).

Fig. 8: The lobed nuclei and the granular cytoplasm of granulocytes becomes easy to recognize at even higher magnification (1,000x, bright field microscopy).

Fig. 9: In the presence of severe leukocyturia single cells become attached to each other to form relatively large clusters (1,000x, bright field microscopy).

Fig. 10: Attachment of white blood cells to hyaline casts or fibers can be confused with white blood cell casts, exhibiting a sign of renal leukocyturia (400x, bright field microscopy).

Fig. 11: In urine white blood cells quickly become attached to other sediment constituents (1,000x, bright field microscopy).

Fig. 12: White blood cells are easily confused with round epithelia. The various cells differ due to typical outgrowths of epithelial cells and the different peripheral cytoplasm (1,000x, bright field microscopy).
Fig. 13: Even at low magnification red blood cells can be differentiated from white blood cells because they are seen as small yellowish dots (100x, bright field microscopy).

Fig. 14: Due to their biconcave shape eumorphic red blood cells appear as sharply delineated ring structures (400x, phase contrast microscopy).

Fig. 15: Red blood cells that have lost their biconcave shape due to osmotic forces appear spherical in phase contrast microscopy (400x, phase contrast microscopy).

Fig. 16: The biconcave shape of eumorphic red blood cells is particularly well visualized when viewed under a scanning electron microscope (courtesy Dr. E. Wandel, Nephrology, University of Mainz).

Fig. 17: Echinocyte in the middle of several biconcave red blood cells (courtesy Dr. E. Wandel, Nephrology, University of Mainz).

Fig. 18: Glomerular hematuria with several acanthocytes and anulocytes (courtesy Dr. E. Wandel, Nephrology, University of Mainz).
Fig. 19: Due to osmotic water emergence red blood cells take on the thorn apple crystal shape of echinocytes (1,000x, bright field microscopy).

Fig. 20: The dysmorphic form changes of red blood cells include hemoglobin-free "ghosts" and shrunken knizocytes as an extreme form of echinocytes (1,000x, bright field microscopy).

Fig. 21: Dysmorphic red blood cells are codocytes and stomatocytes (1,000x, bright field microscopy).

Fig. 22: Red blood cells. Codocytes and stomatocytes are a sign of glomerular hematuria (1,000x, bright field microscopy).

Fig. 23: Eumorphic red blood cell surrounded by "ghost cells", erythrocyte ghosts (1,000x, bright field microscopy).

Fig. 24: In the presence of glomerular hematuria there are acanthocytes (center) and erythrocyte ghosts (“ghost cells”) (1,000x, bright field microscopy).
Fig. 25: Red blood cell casts only occur in hematuria with renal causes (1,000x, bright field microscopy).

Fig. 26: Red blood cell casts in glomerulonephritis (400x, phase contrast microscopy).

Fig. 27: By ingestion of several red blood cells a macrophage has become a giant cell, a red cell phage. This is a rare sign of renal hematuria (1,000x, bright field microscopy).

Fig. 28: This granular cast contains multiple fatty particles, as only occur in severe glomerular proteinuria. Concomitant hematuria is most likely to be of renal origin. The Maltese cross, which is visible in particularly large fatty particles even in bright field microscopy, is characteristic (1,000x, bright field microscopy).

Fig. 29: Multiple granular casts suggest a renal origin of erythrocyturia (400x, bright field microscopy).

Fig. 30: Noticeably, red blood cell casts usually contain eu-morphic red blood cells (1,000x, bright field microscopy).
Fig. 31: In IgA nephropathy, the most frequent form of glomerulonephritis, mixed eumorphic/dysmorphic erythrocyturia is usually found (400x, phase contrast microscopy).

Fig. 32: Vaginal squamous epithelium is characterized by large cells in a small central nucleus and a relatively homogeneous cytoplasm. Multinucleate cells are also observed (400x, phase contrast microscopy).

Fig. 33: In bright field microscopy squamous epithelia can easily be overlooked due to their homogeneous translucent structure (400x, bright field microscopy).

Fig. 34: In phase contrast microscopy, on the other hand, the border structures of squamous epithelial cells are clearly visualized (400x, phase contrast microscopy).

Fig. 35: The borders of squamous epithelia are often folded (400x, phase contrast microscopy).

Fig. 36: Heavily folded squamous epithelia can take on a sickle shape. The concomitant mucosal strands also suggest contamination of the urine with vaginal secretion (400x, phase contrast microscopy).
Fig. 37: Distinct contamination of the urine by vaginal secretion with a large number of squamous epithelia (400x, bright field microscopy).

Fig. 38: Apart from the squamous epithelia, oil globules suggest contamination of the urine by vaginal secretion and skin care products (400x, phase contrast microscopy).

Fig. 39: Fungal hyphae and squamous epithelium in urine as a sign of colpitis (400x, phase contrast microscopy).

Fig. 40: Round epithelia of tubular origin are small circular cells with a medium-size central nucleus (400x, phase contrast microscopy).

Fig. 41: Tubular epithelium (right) differs from white blood cells (left and below) and red blood cells on account of size and different cytoplasmic structure (1,000x, bright field microscopy).

Fig. 42: Although tubular cells (center) can vary in size, differentiation between white blood cells (top) and transitional epithelium (left) is usually possible (1,000x, bright field microscopy).
Microscopic urine sediment

Figures

Fig. 43: Tubular cells (right) are much smaller than transitional epithelium (top right) and squamous epithelium (bottom) (1,000x, bright field microscopy).

Fig. 44: The nucleus size of squamous epithelium (top) and tubular epithelium (center) is approximately the same but the cell types differ due to the size of the cytoplasm (400x, phase contrast microscopy).

Fig. 45: In tubulointerstitial diseases a larger amount of granule-rich round epithelium is found in the urine. Concomitant renal hematuria is also present (1,000x, bright field microscopy).

Fig. 46: The prominent inclusion bodies of the large round epithelial cell suggest primary or secondary tubulointerstitial damage (1,000x, bright field microscopy).

Fig. 47: Tubular cells with many large fatty particles inclusions are termed oval fat bodies and are a sign of severe proteinuria (1,000x, bright field microscopy).

Fig. 48: Degenerative changes in round epithelium are found if the urine is allowed to stand for a lengthy period prior to analysis (1,000x, bright field microscopy).
Fig. 49: The folded borders of this round epithelial cell are a sign of excessively long storage of the urine prior to evaluation (1,000x, bright field microscopy).

Fig. 50: The difference between white blood cells and urothelial cells is their size (1,000x, bright field microscopy).

Fig. 51: Compared to squamous epithelial cells the transitional epithelium of the lower urinary tract and the bladder have a smoothly delineated cell border (1,000x, bright field microscopy).

Fig. 52: With their cytoplasmic branches transitional epithelial cells become firmly attached to each other and to the basement membrane (400x, bright field microscopy).

Fig. 53: Transitional epithelial cells with long thin branches can look roughly like flagella but do not have intrinsic motility (400x, bright field microscopy).

Fig. 54: Some transitional epithelial cells of the deeper layers have longer branches and can easily be confused with cilioids (1,000x, bright field microscopy).
Fig. 55: Transitional epithelia are large circular cells that originate from the lower urinary tract. In the urine they are often eliminated as a cell aggregate (400x bright field microscopy).

Fig. 56: Transitional epithelial cells are eliminated in the urine in a very wide range of forms (400x, phase contrast microscopy).

Fig. 57: Hyaline casts form as a result of precipitation of Tamm-Horsfall protein in the distal tubules of the kidney (400x, hematoxylin-eosin, courtesy Dr. Weis, Pathological Institute, University of Munich).

Fig. 58: Hyaline casts can scarcely be recognized in bright field microscopy (400x, bright field microscopy).

Fig. 59: The same cast is visualized clearly in phase contrast microscopy (400x, phase contrast microscopy).

Fig. 60: By moving the condenser to its uppermost position hyaline casts can also be visualized in bright field microscopy.
Fig. 61: Precipitated Tamm-Horsfall protein does not always have to be homogeneous in hyaline casts (400x, bright field microscopy).

Fig. 62: At higher magnification the different texture at the tip of the hyaline cast is particularly clear (1,000x, bright field microscopy).

Fig. 63: At the head end of this long hyaline cast there are two granulocytes. The tail has a coiled segment and an extended segment (400x, phase contrast microscopy).

Fig. 64: This short hyaline cast has cell inclusions at the tip (400x, phase contrast microscopy).

Fig. 65: In the center segment of this hyaline cast a cell inclusion is found (400x, phase contrast microscopy).

Fig. 66: At higher magnification one can see a fat body cell embedded in the cast as a sign of tubulointerstitial damage (1,000x, bright field microscopy).
Fig. 67: Whether this cast should be described as a hyaline cast with red blood cell inclusions or as a red blood cell cast is not clearly defined. At all events it is a sign of renal hematuria (1,000x, bright field microscopy).

Fig. 68: This hyaline cast with erythrocytic inclusions suggests a renal origin of otherwise eumorphic erythrocyturia (400x, phase contrast microscopy).

Fig. 69: On closer examination this image does not depict erythrocytic inclusions but deposits on a hyaline cast (400x, bright field microscopy).

Fig. 70: In this renal biopsy one can see glomerular damage due to the emergence of serum protein from the capillary loops into the extracapillary space of the glomerulus. From there serum protein enters the tubular lumen, where under the influence of pH and osmolar forces it can precipitate deformed casts (400x, hematoxylin-eosin, courtesy Dr. Weis, Pathological Institute, University of Munich).

Fig. 71: The renal tubules are surrounded by an inflammatory reaction, which causes damage to the tubular cells. Any granular cast found in the urine can be a sign of such damage (400x, hematoxylin-eosin, courtesy Dr. Weis, Pathological Institute, University of Munich).

Fig. 72: Bright field microscopy depicts granular casts as blurred or sharply delineated with an erratic internal structure (400x, bright field microscopy).
Fig. 73: In granular casts glomerular or tubular cell detritus is encapsulated in a Tamm-Horsfall matrix (1,000x, bright field microscopy).

Fig. 74: Shed tubular cells and cytoplasmic cell constituents can be found inside granular casts (1,000x, bright field microscopy).

Fig. 75: The various portions of the casts can have very different sizes of granule, depending on the ratio of Tamm-Horsfall matrix and inclusions (400x, phase contrast microscopy).

Fig. 76: Even relatively long granular casts can be coiled in the rear segment (400x, phase contrast microscopy).

Fig. 77: Granular casts are easy to recognize in bright field microscopy (400x, bright field microscopy).

Fig. 78: Granular casts are noticeable even in an overview, which is why this magnification is more suitable for general orientation regarding the density of casts (100x, bright field microscopy).
Microscopic urine sediment

Figures

Fig. 79: The different surface texture of hyaline casts and granular casts becomes clear under a scanning electron microscope (courtesy Dr. E. Wandel, Nephrology, University of Mainz).

Fig. 80: High-contrast shimmering granular casts are a sign of pigmenturia. In this case chemical analysis or case history must be considered (400x, phase contrast microscopy).

Fig. 81: This is not a granular cast. On closer examination one can see that a hyaline cast is covered by a bacterial lawn (1,000x, bright field microscopy).

Fig. 82: Dark granular casts in the presence of amorphous crystals are usually pseudo casts due to clustering or coated hyaline casts (400x, bright field microscopy).

Fig. 83: Granular casts together with round epithelia suggest tubulointerstitial damage (400x, phase contrast microscopy).

Fig. 84: This cast is likely to be a fatty cast, as is already evident from the hint of a Maltese cross in bright field microscopy (400x, bright field microscopy).
Fig. 85: A calcium oxalate crystal has been deposited on this granular cast from a patient with acute renal failure. It provides no additional diagnostic information (1,000x, bright field microscopy).

Fig. 86: Even in an overview one can recognize multiple pigmented casts and relatively small pigments in a patient with rhabdomyolysis and acute renal failure (soiled sediment) (100x, bright field microscopy).

Fig. 87: In a granular cast with its pigmented color no red blood cells are enclosed in the matrix. Nor were any other signs of erythrocyturia found (1,000x, bright field microscopy).

Fig. 88: The head segment of this yellow-brown granular cast contains plenty of yellow pigment but no red blood cells (1,000x, bright field microscopy).

Fig. 89: The amorphous crystals next to the yellow-brown cast are not associated with the cause of acute renal failure (400x, phase contrast microscopy).

Fig. 90: Stained granular casts can also be reliably differentiated from contaminations (400x, bright field microscopy).
Microscopic urine sediment

Figures

**Fig. 91:** Waxy casts are wide homogeneous casts with a sharp border and rounded corners (1,000x, bright field microscopy).

**Fig. 92:** Lateral notches are typical of waxy casts (400x, phase contrast microscopy).

**Fig. 93:** Waxy casts occasionally have a spiral structure in the tail segment (400x, phase contrast microscopy).

**Fig. 94:** This waxy cast is slightly inhomogeneous and has a spiral tail. In the transitional segment between the body and tail one can see how the lateral notches form due to the compressed spiral structure (400x, bright field microscopy).

**Fig. 95:** Occasionally, waxy casts form from a strand of granular matrix and a strand of hyaline matrix (400x, bright field microscopy).

**Fig. 96:** Spirals in the fragment of a waxy cast (400x, bright field microscopy).
Fig. 97: A granular cast is surrounded by a wax layer (1,000x, bright field microscopy).

Fig. 98: A fragment of a waxy cast that has a completely homogeneous texture at one end and a granular texture at the other (400x, bright field microscopy).

Fig. 99: Red blood cell casts develop in a Tamm-Horsfall matrix in renal tubules (hematoxylin-eosin, 1,000x, courtesy Dr. Weis, Pathological Institute at the University of Munich).

Fig. 100: With minimal glomerular bleeding there are sometimes only isolated red cell inclusions in hyaline casts (1,000x, bright field microscopy).

Fig. 101: In this long thin cast one can see how several red blood cells are enclosed in a hyaline matrix (1,000x, bright field microscopy).

Fig. 102: These short red blood cell casts only appear to consist of compressed red blood cells (1,000x, bright field microscopy).
Fig. 103: In the upper section of the image one can see an acanthocyte. Within the cast, however, almost only eumorphic red blood cells can be found (1,000x, bright field microscopy).

Fig. 104: In addition to a granular structure this cast contains red blood cells, white blood cells, and a still recognizable epithelial cell. The yellowish color is hemoglobin that has emerged (1,000x, bright field microscopy).

Fig. 105: This mixed cell cast also consists of red blood cells, epithelial cells, and white blood cells (1,000x, bright field microscopy).

Fig. 106: At the yellowish colored head end of this wide granular cast one can also recognize individual red blood cell contours (1,000x, bright field microscopy).

Fig. 107: This red blood cell cast contains two tubular cells (1,000x, bright field microscopy).

Fig. 108: In a patient with acute renal failure this epithelial cell cast was found along with single red cell inclusions (1,000x, bright field microscopy).
Fig. 109: In this red blood cell/hemoglobin cast one can see how burst red blood cells release hemoglobin so cell contours can no longer be recognized with certainty (1,000x, bright field microscopy).

Fig. 110: This mixed cell cast contains single white blood cells and tubular cells. There is a tubular cell alongside (1,000x, bright field microscopy).

Fig. 111: In this cast with a granular matrix there are several white blood cell inclusions (1,000x, bright field microscopy).

Fig. 112: This long hyaline cast also contains single white blood cells as a sign of inflammatory renal disease (400x, bright field microscopy).

Fig. 113: In a typical white blood cell cast the cells are close to each other in a hyaline matrix (1,000x, bright field microscopy).

Fig. 114: Pseudo cast. In severe leukocyturia clusters of white blood cells can be confused with white blood cell casts (1,000x, bright field microscopy).
Fig. 115: In this mixed cell cast the borders of white blood cells and tubular epithelium can scarcely be delineated (1,000x, bright field microscopy).

Fig. 116: In acute tubular necrosis there are typically double rows of tubular epithelium in cast form (1,000x, bright field microscopy).

Fig. 117: In this pure epithelial cell cast the cell borders can no longer be clearly delineated (1,000x, bright field microscopy).

Fig. 118: Epithelial cell cast and a finely granular cast in a patient with shock kidneys (400x, phase contrast microscopy).

Fig. 119: Erythrocyturia and an epithelial cell cast (400x, bright field microscopy).

Fig. 120: The typical cell structure of tubular cells is still easy to recognize in this epithelial cell cast (1,000x, bright field microscopy).
Fig. 121: Sometimes single tubular cells are enclosed in a hyaline cast, even in subjects with healthy kidneys (400x, phase contrast microscopy).

Fig. 122: In this cast as well with a hyaline matrix one can see enclosed tubular cells (1,000x, bright field microscopy).

Fig. 123: In the head segment of this hyaline casts there are several epithelial cell inclusions (1,000x, bright field microscopy).

Fig. 124: In this small fatty cast the fatty particles are crowded close to each other. The small fatty particles have a much higher refractive index and a darker color than red blood cells (400x, bright field microscopy).

Fig. 125: In dark field microscopy one can recognize the shining fat bodies and the typical Maltese cross (400x, dark field microscopy).

Fig. 126: In this fatty cast there are also relatively large fatty particles (1,000x, dark field microscopy).
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Figures

Fig. 127: In a patient with eumorphic erythrocyturia this long hyaline cylindroid was first noticed in phase contrast microscopy (400x, phase contrast microscopy).

Fig. 128: Hyaline cylindroid with an epithelial cell inclusion as a sign of origin in the kidney (400x, phase contrast microscopy).

Fig. 129: Granular cylindroid with a wide head and a thin tail segment (400x, phase contrast microscopy).

Fig. 130: In a patient with glomerulonephritis there was not only dysmorphic erythrocyturia but also a cylindroid with a hemoglobin-containing head as a sign of renal hematuria (400x, phase contrast microscopy).

Fig. 131: Granular cylindroid in acute tubular necrosis (400x, phase contrast microscopy).

Fig. 132: Granular cylindroid (400x, bright field microscopy).
Fig. 133: Staphylococcal cast in endocarditis with septic focal nephritis (1,000x, bright field microscopy).

Fig. 134: In this bacterial cast it is difficult to differentiate between bacterial inclusions and deposits. The squamous epithelia suggest contamination with genital secretion (1,000x, bright field microscopy).

Fig. 135: Yeast cells have been enclosed in the tubules of the kidney in a hyaline matrix. Leukocyturia also supports the finding: fungal cast (1,000x, bright field microscopy).

Fig. 136: This yeast cell cast surrounded by white blood cells was found in the urine of a patient with fungemia (1,000x, bright field microscopy).

Fig. 137: This calcium oxalate cast in a patient with symptomatic hypercalciuria is not unlike a red blood cell cast (1,000x, bright field microscopy).

Fig. 138: This cast consists entirely of calcium oxalate dihydrate. It does not shine in polarized light. Relatively small crystals can be seen inside (1,000x, polarized light).
Fig. 139: Urate nephropathy. Small uric acid crystals are also enclosed in this white blood cell cast, which documents a renal origin (1,000x, polarized light).

Fig. 140: Uric acid crystals and an epithelial cell in the head of a hyaline cast. In addition there are yeast cells and white blood cells on a massive scale (1,000x, polarized light).

Fig. 141: Small uric acid crystals become attached to the outside of a mucosal strand (400x, bright field microscopy).

Fig. 142: This pseudo cast is formed by calcium phosphate crystals (1,000x, polarized light).

Fig. 143: Pseudo casts are often found in the urine sediment of patients with eumorphic macroscopic hematuria. Hyaline border delineation is not even possible in phase contrast microscopy (400x, bright field microscopy).

Fig. 144: In this case a cast is evident from a layer of crystals and red blood cells. Pseudo cast in a patient with bladder carcinoma (400x, phase contrast microscopy).
Fig. 145: Hair and air bubble. The highly birefringent edge of the hair and the split ends allow certain delineation of a hyaline cast (400x, bright field microscopy).

Fig. 146: This hair served as a seed for uric acid crystals (400x, bright field microscopy).

Fig. 147: At higher magnification one can see the crystal deposits (1,000x, bright field microscopy).

Fig. 148: Foreign matter as a pseudo cast (400x, bright field microscopy).

Fig. 149: Trichomonads are either round or oval and can easily be confused with white blood cells or epithelial cells (1,000x, bright field microscopy).

Fig. 150: Squamous epithelia suggest contamination with vaginal secretion in a patient with trichomonas colpitis (1,000x, bright field microscopy).
Fig. 151: A trichomonas infection can initially be falsely diagnosed as sterile leukocyturia. Intrinsic motility in fresh urine is significant (400x, bright field microscopy).

Fig. 152: At low magnification yeasts cannot be differentiated from red blood cells (100x, bright field microscopy).

Fig. 153: Only at higher magnification is it possible to recognize the typical shape of the yeasts with their classic budding (1,000x, bright field microscopy).

Fig. 154: If candiduria is accompanied by leukocyturia, an infection exists that requires therapy (400x, bright field microscopy).

Fig. 155: Vegetative yeast forms can be recognized from their glassy interior with a central nucleolus (1,000x, bright field microscopy).

Fig. 156: Budding yeasts, on the other hand, have a central bright spot. They are accompanied by erythrocyturia, “ghost cells”, and leukocyturia (1,000x, bright field microscopy).
Fig. 157: Contamination of the urine with squamous epithelium and budding yeasts (400x, phase contrast microscopy).

Fig. 158: Yeast mycelium with proliferous blastocytic sprouting (1,000x, bright field microscopy).

Fig. 159: Squamous epithelial cells and long fungal hyphae were found in this contaminated urine sample (100x, bright field microscopy).

Fig. 160: In rare cases relatively unusual yeast forms are also detected in the urine (1,000x, bright field microscopy).

Fig. 161: Short fungal cast in a patient with fungemia. Concomitant leukocyturia and bacteriuria (1,000x, bright field microscopy).

Fig. 162: Rod-shaped bacteria can best be seen in bright field microscopy with the condenser in a high position. Red blood cells and white blood cells suggest a hemorrhagic urinary tract infection (1,000x, bright field microscopy).
Fig. 163: Extensive bacterial growth is found especially in urine samples that were not fresh when tested (400x, bright field microscopy).

Fig. 164: Cocci can also be recognized in bright field microscopy at high magnification (1,000x, bright field microscopy).

Fig. 165: Bacteria become attached to or grow over other sediment constituents such as casts and epithelial cells (400x, bright field microscopy).

Fig. 166: Pyuria, bacteriuria, and epithelial cells are signs of contamination with vaginal secretion in colpitis. Naturally a urinary tract infection can also be present (1,000x, bright field microscopy).

Fig. 167: In the urine of a patient with endocarditis lenta there were tubular epithelia, white blood cells, and cocci. Diagnosis: septic focal nephritis (1,000x, bright field microscopy).

Fig. 168: Such bacterial casts can be found in ascending urinary tract infections (1,000x, bright field microscopy).
Fig. 169: No matter whether they are small or large, calcium oxalate crystals resemble sparkling diamonds. Here they are accompanied by some epithelial cells. At the center there is a circular urate crystal (400x, phase contrast microscopy).

Fig. 170: Single calcium oxalate crystals can become very large. Here there is a symmetric tetramer (400x, bright field microscopy).

Fig. 171: Longish forms of calcium oxalate are found more rarely (400x, bright field microscopy).

Fig. 172: Calcium oxalate occurs in small round crystals or hourglass forms of various sizes. They shine in colors in polarized light. At the center there is a granular cast with an enclosed epithelial cell (400x, polarized light).

Fig. 173: In this section calcium oxalate dihydrates and monohydrates are next to each other. The monohydrates shimmer in colors in bright field microscopy (1,000x, bright field microscopy).

Fig. 174: In dark field microscopy the refringent property of calcium oxalate monohydrates is particularly well visualized (1,000x, dark field microscopy).
Fig. 175: Calcium oxalate monohydrates in an hourglass form. Accompanied by leukocyturia (1,000x, bright field microscopy).

Fig. 176: Oval calcium oxalate monohydrate crystals shine in colors when exposed to polarized light (400x, polarized light).

Fig. 177: Various shapes and sizes of calcium oxalate monohydrate crystals (400x, bright field microscopy).

Fig. 178: In an overview one can see non-refringent calcium oxalate dihydrates and small oval monohydrates that already shine in colors in bright field microscopy (100x, bright field microscopy).

Fig. 179: Small circular calcium oxalate monohydrates can easily be confused with red blood cells (400x, phase contrast microscopy).

Fig. 180: In bright field microscopy one can recognize uric acid crystals on account of their highly refringent properties. The forms, on the other hand, can vary considerably (1,000x, bright field microscopy).
Fig. 181: Shining in colors when exposed to polarized light is characteristic (1,000x, polarized light).

Fig. 182: Barrel-shaped uric acid crystals (1,000x, bright field microscopy).

Fig. 183: Rhombic uric acid crystals, which can also be very large, are typical (400x, polarized light).

Fig. 184: Small circular uric acid crystals can easily be confused with red blood cells (1,000x, bright field microscopy).

Fig. 185: In this air bubble there are six biconcave uric acid crystals. In polarized light it is easy to differentiate them from red blood cells (1,000x, polarized light).

Fig. 186: In this contaminated urine sample sediment constituents provide the seed for the formation of uric acid crystals in various shapes (400x, phase contrast microscopy).
Fig. 187: Amorphous urates are small dark grains that give sediment a soiled appearance. In between there are calcium oxalate dihydrate crystals (100x, bright field microscopy).

Fig. 188: Amorphous urate darkens other sediment constituents (400x, bright field microscopy).

Fig. 189: In urate nephropathy urate casts and amorphous urate are found (1,000x, bright field microscopy).

Fig. 190: A calcium phosphate plate is deposited on small amorphous phosphates (400x, bright field microscopy).

Fig. 191: Highly refringent calcium phosphate crystal (400x, phase contrast microscopy).

Fig. 192: This fragmented calcium phosphate crystal resembles glass particles (400x, bright field microscopy).
Fig. 193: Scaly calcium phosphates agglomerate to create larger formations (400x, phase contrast microscopy).

Fig. 194: Finely granular grayish calcium phosphate plate (400x, phase contrast microscopy).

Fig. 195: Fragmented finely granular calcium phosphate plate (400x, bright field microscopy).

Fig. 196: Calcium carbonate crystals can also be present in small dumbbell forms (400x, phase contrast microscopy).

Fig. 197: Amorphous calcium carbonate makes it difficult to evaluate diagnostically important structures (100x, bright field microscopy).

Fig. 198: Erythrocyturia and colored magnesium ammonium phosphate crystals (100x, polarized light).
Fig. 199: The trapezoidal shape of magnesium ammonium phosphates causes the envelope form due to translucent edges (1,000x, bright field microscopy).

Fig. 200: The symmetry and colors of magnesium ammonium phosphates are particularly attractive (400x, polarized light).

Fig. 201: In alkaline urine magnesium ammonium phosphate crystals and amorphous phosphate are found (400x, bright field microscopy).

Fig. 202: Magnesium ammonium phosphate can occasionally also form long rod crystals. The crystals are surrounded by amorphous phosphate. Both are a sign of alkaline urine pH (400x, phase contrast microscopy).

Fig. 203: Dicalcium phosphate crystals usually take the form of druses (1,000x, bright field microscopy).

Fig. 204: Dicalcium phosphate and amorphous phosphate (400x, bright field microscopy).
Fig. 205: Alkaline urine occasionally contains large, translucent dimagnesium phosphate plates (400x, bright field microscopy).

Fig. 206: The sharp-edged dimagnesium phosphates usually have a thick border and a thin border, which is why they resemble glass splinters (1,000x, bright field microscopy).

Fig. 207: Dimagnesium phosphates are sometimes quite flat (400x, bright field microscopy).

Fig. 208: Some dimagnesium phosphates are slim and long (400x, bright field microscopy).

Fig. 209: Slim dimagnesium phosphates can be confused with needle-shaped drug crystals (400x, phase contrast microscopy).

Fig. 210: Tyrosine crystals are small needles that usually form rosettes. Here they are accompanied by erythrocyturia (400x, phase contrast microscopy).
Fig. 211: Typical leucine crystal and leukocyturia (1,000x, polarized light).

Fig. 212: Leucine crystals are occasionally colored even in bright field microscopy (1,000x, bright field microscopy).

Fig. 213: In dark field microscopy the highly refringent property of leucine crystals is particularly evident (1,000x, dark field microscopy).

Fig. 214: Cystine crystals usually occur as flat hexagonal plates that are colorless (courtesy Dr. E. Wandel, Nephrology, University of Mainz, 400x, phase contrast microscopy).

Fig. 215: More rarely one can see small rosettes of cystine crystals (400x, phase contrast microscopy).

Fig. 216: Ammonium biurate spheres can scarcely be confused (400x, bright field microscopy).
Fig. 217: In a patient with chronic polyarthritis this symptomatic crystalluria was present as a complication of sulfasalazine therapy (1,000x, bright field microscopy).

Fig. 218: For the treatment of pneumocystis carinii pneumonia high doses of sulfasalazine-trimetoprim are used. Crystalluria can also occur (400x, bright field microscopy).

Fig. 219: Crystalluria is rare in the presence of vancomycin (400x, polarized light).

Fig. 220: In the case of proteinase inhibitors for the treatment of HIV infection crystalluria particularly occurs in the presence of indinavir. Concomitant erythrocyturia existed in a patient with postrenal kidney failure with bilateral ureteral occlusion by crystal concretions (100x, bright field microscopy).

Fig. 221: Indinavir crystals at high magnification (400x, bright field microscopy).

Fig. 222: When exposed to polarized light indinavir crystals shine in all colors (400x, polarized light).
Fig. 223: Single large fat globules are recognized by a shimmering Maltese cross. Alongside there is an oval fat body consisting of numerous small lipid particles (1,000x, bright field microscopy).

Fig. 224: Oval fat body with numerous small and large fat globules (1,000x, bright field microscopy).

Fig. 225: In a patient with nephrotic syndrome there were small free fat globules, but also numerous oval fat bodies (400x, phase contrast microscopy).

Fig. 226: Tubular cell with vacuolar cytoplasm in a patient with severe proteinuria in membranous glomerulonephritis (400x, phase contrast microscopy).

Fig. 227: This small crystal and dark crystal in a patient with minimal change glomerulonephritis are cholesterol plates. Erythrocyturia is present also (400x, phase contrast microscopy).

Fig. 228: Cholesterol crystal in nephrotic syndrome and "active nephritic" sediment (400x, phase contrast microscopy).
Fig. 229: Sperms can occasionally be found in the urine of healthy subjects (400x, bright field microscopy).

Fig. 230: Maturation disorders of spermatozoa are seen following chemotherapy (1,000x, bright field microscopy).

Fig. 231: In bright field microscopy the mucosal strands of vaginal secretions are not clearly visible (1,000x, bright field microscopy).

Fig. 232: In phase contrast microscopy, on the other hand, mucosal strands are visualized very clearly (400x, phase contrast microscopy).

Fig. 233: Small calcium carbonate crystals become attached to sticky vaginal mucus (400x, phase contrast microscopy).

Fig. 234: However, in contaminated urine samples a red blood cell cast suggests a renal cause of hematuria (400x, phase contrast microscopy).
Fig. 235: Hairs in urine are easy to recognize from their substantial refraction and frayed ends (400x, bright field microscopy).

Fig. 236: Hairs can scarcely be confused with hyaline casts (400x, phase contrast microscopy).

Fig. 237: Large oil particles usually originate from skin care products (400x, phase contrast microscopy).

Fig. 238: At low magnification talcum particles can easily be confused with cellular structures (100x, bright field microscopy).

Fig. 239: Even at high magnification the differentiation of uric acid crystals, fat globules, and red blood cells is not always easy (1,000x, bright field microscopy).

Fig. 240: In dark field microscopy the distorted Maltese cross of talcum particles is characteristic though (1,000x, dark field microscopy).
Fig. 241: Meat fibers prove fecal contamination of the urine (100x, bright field microscopy).

Fig. 242: White blood cells become attached to material fibers (1,000x, bright field microscopy).

Fig. 243: Fiber particles form the seed for magnesium ammonium phosphate (100x, polarized light).

Fig. 244: Pollen particles can be found in many urine samples in early summer (1,000x, bright field microscopy).

Fig. 245: Pollen particles can be confused with ammonium biurate spheres (400x, bright field microscopy).

Fig. 246: Air bubble and other foreign matter (400x, phase contrast microscopy).
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Figures

Fig. 247: Talcum particles can initially be confused with amorphous material or fatty particles (1,000x, bright field microscopy).

Fig. 248: Even in phase contrast microscopy talcum particles resemble crystals (400x, phase contrast microscopy).

Fig. 249: However, the distorted Maltese cross becomes visible in polarized light (1,000x, polarized light).

Fig. 250: In talcum particles the distorted cross shines in dark field microscopy (1,000x, dark field microscopy).

Fig. 251: Active sediment with dysmorphic red blood cells and acanthocytes (1,000x, bright field microscopy).

Fig. 252: Erythrocyturia and red blood cell casts in acute glomerulonephritis (1,000x, bright field microscopy).
Fig. 253: White blood cells and bacteria on a massive scale are important signs of a urinary tract infection (1,000x, bright field microscopy).

Fig. 254: If white blood cells casts and epithelial cell casts are also found, clinical suspicion of pyelonephritis can be confirmed (1,000x, bright field microscopy).

Fig. 255: This urine sample originates from a female patient with membranous glomerulonephritis. One can see hyaline casts, erythrocyturia, and a cholesterol crystal as a sign of severe proteinuria (400x, phase contrast microscopy).

Fig. 256: Dysmorphic erythrocyturia and a highly vacuolated tubular epithelial cell in the urine of a patient with focal sclerosing glomerulopathy (1,000x, bright field microscopy).

Fig. 257: Fatty casts in IgA nephropathy with severe proteinuria (1,000x, bright field microscopy).

Fig. 258: The same cast in dark field microscopy with the typical sign of fatty particles (1,000x, dark field microscopy).
Fig. 259: Epithelial cell casts are typical of tubulointerstitial renal diseases (1,000x, bright field microscopy).

Fig. 260: This sediment contains granular casts, amorphous phosphates, and drug crystals as a sign of toxic renal failure (400x, phase contrast microscopy).

Fig. 261: Mixed cell casts are particularly found in tubulointerstitial renal diseases (1,000x, bright field microscopy).

Fig. 262: Granular casts and erythrocyturia in a patient with shock kidneys (1,000x, bright field microscopy).

Fig. 263: Mixed cell cast in acute tubular necrosis (1,000x, bright field microscopy).

Fig. 264: Yellow-brown cast in myoglobinuria (1,000x, bright field microscopy).
Fig. 265: Bacteria and white blood cells in bacterial cystitis (400x, bright field microscopy).

Fig. 266: White blood cells and bacteria on a massive scale as a sign of urinary tract infection (1,000x, bright field microscopy).

Fig. 267: Cocci and white blood cells suggest contamination or hematogenic spread (1,000x, bright field microscopy).

Fig. 268: Squamous epithelial cells and long chains of rod-shaped bacteria as a sign of contamination (400x, bright field microscopy).

Fig. 269: Squamous epithelial cells, bacteria, and white blood cells suggest colpitis (400x, bright field microscopy).

Fig. 270: White blood cells and yeasts without squamous epithelium, from a diabetic in this case, mean that urinary tract infection is likely (1,000x, bright field microscopy).
Fig. 271: The short fungal hypha is barely visible between the white blood cells (1,000x, urinary tract infection).