Ministry of Health of Ukraine
Kharkiv National Medical University

URINE ANALYSIS, RENAL FUNCTION TESTS

Methodical instructions for students

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The investigation of the patient with suspected renal disease usually requires assessment of both structure and function of the kidneys. Structural evaluation includes imaging studying and

microscopic examination of tissue samples acquired by biopsy. Function is assessed by examination of urine and biochemical determinations on plasma samples.

URINE ANALYSIS

Clinical urine analysis includes: macroscopic (physical properties), chemical, microscopic, bacteriological and bacterioscopic studies.

Collection of the urine

Urine sample is taken after night sleep in the clear and dry container. Urine sample can be collected from males after retraction of the foreskin, and from females with the labia separated by their fingers. Ideally the genitalia should be swabbed with sterile saline but this is often impracticable. Antiseptics should be avoided if the sample is required for culture. The urine sample must be sent promptly to the laboratory to avoid growth of contaminant organisms and the dissolution of cellular elements and casts. It should be cooled in refrigerator at 4˚C if delay is likely to be greater than 2 h.

Macroscopic study

Macroscopic study includes assessment of physical properties of the urine: amount, color, cloudiness, smell, and specific gravity.

Physical properties of the urine

Amount of the urine. In healthy adult the normal amount of excreted urine is between 1000 ml and 2000 ml in 24 h (diurnal diuresis).

Polyuria is defined as a production by an adult of more than 2000 ml of urine/24h.

Olyguria is defined as a production by an adult of less than 500 ml of urine/24h.

Color of the urine depends on the presence of physiologic pigments (urochromes, urobilinoids, uroerythrin, etc) and on its concentration. The color of the normal urine varies from straw yellow to orange-yellow. Different pathological conditions of the urinary organs can cause peculiar changes of the urine color (Tab. 8).

Tab. 1. Clinical significance of urine color changes.

Color	Pathological condition	Cause
Dark yellow	Congestive kidney, edema, burns, diarrhea, vomiting	High concentration of urochrome
Pale, water-like	Diabetes mellitus, diabetes insipidus	Low concentration of urochrome
Dark	Hemolytic anemia	Urobilinogenuria

Dark, almost black	Acute hemolytic kidney	Hemoglobinuria
Red	Renal colic, renal infarction	Hematuria (unaltered blood)
Appearance of "meat wastes"	Acute glomerulonephritis	Hematuria (altered blood)
Greenish-brown (beer-like)	Parenchymatous jaundice	Bilirubunuria, urobilinuria
Greenish-yellow	Obstructive jaundice	Bilirubinuria
Whitish	Fatty degeneration and decomposition of the renal tissue	Lipuria
Milky	Renal lymphostasis	Hyluria

Cloudiness of the urine. Normal, freshly excreted urine is clear. Cloudiness of the urine can be cause by the presence of salts, cellular elements (leucocytes, erythrocytes, epithelium cells), bacteria, mucus, and fats.

Smell of the urine. Normally, the urine has not strong specific smell. In bacterial decomposition on air or in urinary ducts (severe cystitis, degradation of malignant tumor) urine smells of ammonia. Peculiar "fruity" or "apple" odor of the urine is characteristic of diabetic coma or diabetes mellitus in decompensation stage. Such specific odor of the urine is a result of ketone bodies presence.

Specific gravity of the urine is proportional to concentration of dissolved in it substances: urea, uric acid, various salts, and depends not only on amount but mainly on their molecular weight.

Specific gravity is measured by urometer, normally it varies from 1.015 to 1.025. In health there is diurnal variation of the specific gravity; in morning, the most concentrated portion of the urine, it can be to 1.020-1.026.

Assessment of the specific gravity of the urine is of great diagnostic significance, because these parameter gives information about concentrating ability of the kidneys. The specific gravity can also be depends on the volume of urine excreted (Tab. 2).

Tab. 2. Clinical significance of specific gravity changes.

Specific gravity	Extrarenal causes		Renal causes
	Physiological	Pathological	
Low	Polyuria	Diuretics taking	Renal failure

		Alimentary dystrophy Diabetes insipidus	
High	Oliguria	Fluid accumulation in cavities and tissues (edema, ascitis, hydrothorax, etc) Profuse vomiting, diarrhea Diabetes mellitus	Renal glucosuria Renal amyloidosis with high proteinuria

Zimnitsky's test characterize condition of renal concentrating and excretory ability. In order to correct measure urinary concentrating ability, the patient must avoid taking much fluid.

Urine samples are collected each 3 hours in separate container with designation of time – 8 portions during 24 hours. Volume and specific gravity of the urine is measured in each portion.

The advantages of this method are:

- Possibility to measure diurnal diuresis and to detect presence of polyuria or oliguria;
- Possibility to measure separately daily and nightly diuresis and to detect presence of nicturia;
- Possibility to determine diurnal variation of the specific gravity and its maximal value.

Normally, diurnal diuresis is 1000-2000 ml, amount of urine in each portion can vary from 50 to 250 ml, daily diuresis exceeds nocturnal, and specific gravity vary from 1.010 to 1.025. if the maximal mean of specific gravity in Zimnitsky's test exceeds 1.020, renal concentrating ability is considered to be normal.

Low specific gravity in all portions is typical to renal failure.

Isosthenuria is defined as condition when osmotic concentration of urine is equal to osmotic concentration of blood plasma. Maximal osmotic concentration of urine in isosthenuria is 270-330 mmol/l, and maximal specific gravity – 1.010-1.012.

Hyposthenuria is defined as condition when maximal osmotic concentration of urine is less than osmotic concentration of blood plasma. Maximal osmotic concentration of urine in hypusthenuria is 200-250 mmol/l, and specific gravity of urine – 1.005-1.008.

Extrarenal causes of urine specific gravity changes

In diabetes mellitus, polyuria and high specific gravity of the urine (to 1.026-1.050) due to glucosuria is determined.

Diabetes insipidus and pituitary insufficiency are characterized by polyuria and low specific gravity of the urine.

Renal causes of urine specific gravity changes

In acute glomerulonephritis, nephrotic syndrome, and in congestive kidney in heart failure osmotic concentration of urine is elevated to 1200 mosm/l, specific gravity of the urine – to 1.031-1.035, that accompanied by oliguria. Hyposthenuria in normal diuirnal diuresis and nicturia observe in patients with chronic glomerulonephritis, chronic pyelonephritis, and nephrosclerosis. Isosthenuria suggests complete absence of renal concentrating ability. Long standing excretion of urine with low specific gravity, monotonous means in combination with oliguria are the signs of severe chronic renal failure with unfavorable prognosis.

Chemical study

Chemical study includes assessment of reaction of the urine (urine pH), protein, glucose, ketone bodies, and bile pigments.

Reaction of the urine – urine pH can be determined calorimetrically (litmus paper and other indicators) and electrometrically.

The urine reaction may vary from pH 5.0 to 7.0 – neutral or feebly acid reaction. Urine pH can be changed in both physiological and pathological conditions (Tab. 3).

Tab. 3. Clinical significance of urine pH changes.

Urine reaction		
Acid	Neutral Feebly acid	Alkaline
Physiological conditions: much meat food intake *Pathological conditions:* diabetes mellitus, severe renal failure, acute nephritis, congestive kidney, tuberculosis of the kidneys, acidosis, hypokaliaemic alkalosis	Norm	*Physiological conditions:* vegetable diet, at the height of digestion, ample alkaline fluid intake *Pathological conditions:* Vomiting, diarrhea, chronic infections of the urinary tracts

Protein. The normal amount of protein excreted in the urine per 24 hours is 25-75 mg that cannot be detected by routine tests. More than half of this amount consists of small molecular weight proteins or protein fragments, although albumin is the largest single component.

Proteinuria is the appearance of protein in the urine in cincentration determinable by qualitative methods.

The protein content of the urine of normal individuals can rise to about 150 mg/l when the urine is concentrated.

Selective and non-selective proteinuria is distinguished. *Selective proteinuria* is characterized by the presence in the urine of low molecular weight proteins – albumin, ceruloplasmin, and transferrin. In non-selective proteinuria high molecular weight proteins – α_2-macroglobulib, β_2-lipoprotein, and γ-globulin are detected. Moreover, Bence-Jones proteins – low molecular weight proteins, can be revealed in the urine. In some pathological conditions, hemoglobin, hemosiderin, myoglobin, and Tamm-Harsfall proteins are present in the urine.

Depend on protein- amount in the urine, *microalbuminuria* – 30-300 mg/24h, and *proteinuria (macroalbuminuria)* – more than 300 mg/24h are distinguished.

Proteinuria can be functional and organic. *Functional proteinuria* observed in subjects without renal diseases, has transitory character, does not exceeds 1 g/24h, and are not accompanied by the other urine abnormalities. Postural (orthostatic), effort, and cold proteinuria are differentiated. Healthy adults are found to have proteinuria when up and about, but not after a period of horizontal rest. Standing position can induce significant proteinuria in a substantial proportion of people who do not otherwise show it. Proteinuria can also be observed in subjects without renal diseases after severe exercise, in fever, or on exposure to extremes of cold or heat. These findings do not imply the presence of renal disease and do not require further investigation.

Organic proteinuria can came about in three ways:

1. The glomerular filter becomes more permeable to proteins of large molecular size, as well as permitting those of small molecular weight to pass – ‘glomerular’ proteinuria. This is by far the commonest cause of proteinuria in clinical practice.
2. There is a marked rise in the plasma concentration of protein in circulation, so that amount filtered exceeds the reabsorptive capacity of the proximal tubule – ‘overflow’ proteinuria.
3. The proximal tubule is damaged so that normally reabsorbed proteins, principally of low molecular weight, pass into the urine – ‘tubular’ proteinuria (Tab. 4).

Tab. 4. Clinical significance of renal proteinuria.

Types	Renal		
	Glomerular		Tubular
Character	Transitory	Constant	Transitory
Protein content	Albumin γ-globulin lisocin	Albumin Transferrin α_2-macroglobulin γ-globulin	Albumin α_2-macroglobulin β_2-microglobulin
Pathological condition	Acute infectious diseases, enteritis, colitis, trauma, burns,liver diseases, diabetic coma, cerebral stroke	Acute and chronic glomerulonephritis, renal amyloidosis, diabetic nephropathy, pyelonephritis, renal veins thrombosis, rheumatic arthritis, congestive kidney, end-stage renal diseases.	Acute tubular necrosis, interstitial nephritis, genetic tubulopathy, Fankoni syndrome

Extrarenal proteinuria is usually caused by protein admixtures (inflammatory exudates, degradated cells) in the diseases of urinary and sex ducts. Such proteinuria usually does not exceed 1g/l.

Glucose. Excretion of glucose with urine is called glycosuria. Glucose is freely filtered by the glomerulus and reabsorbed actively by the proximal tubule. Under normal circumstances, reabsorption is complete but if the blood glucose rises sufficiently, a plasma level is reached (the threshold) at which the transport mechanism is saturated and glucose starts to spill into the urine. Glycosuria could arise in principle in two different ways. First, if the plasma glucose concentration rises above the threshold level (around 10 mmol/l in man) the unreabsorbed glucose will appear in the urine, and this occurs in uncontrolled diabetes mellitus, the commonest clinical cause of glycosuria. Alternatively, if the tubular mechanism for reabsorption is defective, glucose will appear in the urine even when the plasma glucose is within the normal range. This can occur as a result of an inherited abnormality in the protein mediating glucose transport across the proximal tubular cells, or as a consequence of a disease process interfering with the function of this epithelium, as in cyctinosis or tubular damage by heavy metals and other toxins.

Glycosuria can be physiological and pathological (Tab.5).

Tab. 5. Clinical significance of glycosuria.

Physiological	Pathological
Alimentary (carbohydrates diet) Following emotional stress Taking of some medicines: caffeine, corticosteroids, epinephrine	Renal diabetes as a primary disease (blood glucose is normal) Secondary renal glycosuria in chronic nephritis and nephrotic syndrome Diabetes mellitus Thyrotoxicosis Pituitary insufficiency (Itsenko-Cushing disease), Liver cirrhosis

Physiological glycosuria is usually transitory, and pathologic – constant.

Ketone bodies (acetone, acetoacetic and β_2-oxybutyric acid) are normally absent in the urine. Ketonuria is defined as a presence on ketone bodies in the urine. They usually occur in diabetes mellitus, carbohydrate deficit: fasting, grave toxicities, long- standing intestinal disorders, dysentery, and in postoperative period. Ketonuria is important laboratory sign of decompensation of diabetes mellitus with transformation to diabetic coma.

Bilirubin. Normal urine contains minimal quantity of the bilirubin. Increased excretion of bilirubin is pathological condition and is called – bilirubinuria. Bilirubinuria occurs in increased blood level of bound bilirubin more than 0,01-0,02 g/l ("renal threshold of bilirubin") in parenchymatous jaundice (acute virus, toxic, toxico-allergic hepatitis, liver cirrhosis), subhepatic jaundice (altered permeability of bile ducts due to inflammation, obstruction by stones, by tumor, or by scars).

Urobilinoids: urobilin (urobilinogens, urobilins) and stercobilin (stercobilinogens, stercobilins) are derivates of bilirubin. They are not determined separately. A large quantity of urobilinoids in urine is called urobilinogenuria. It occurs mainly in: parenchymatous affection of the liver (hepatitis, cirrhosis), hemolytic processes (hemolytic anemia); and in intestinal diseases (enteritis, constipation, etc).

Macroscopic study

Erythrocytes. The urine of healthy person contains single erythrocytes. The presence of erythrocytes in the urine is called haematuria. Determination of erythrocytes in microscope vision area (more than 1000 cells in 1 ml) is defined as microhaematuria; the color of the urine is unchanged in such cases. If erythrocytes amount is 2500

cells in 1 ml, the urine is of red color that is defined as macrohaematuria.

Haematuria can be true (from the kidneys and urinary tract) and false (in man in prostatitis, tuberculosis and tumor of prostate, in woman of genitalia origin).

Erythrocytes in the urine can be altered and unaltered depend on their origin (Tab. 6).

Tab. 6. Clinical significance of haematuria.

Haematuria	
Glomerular origin	Non glomerular origin
Altered erythrocytes • Acute nephritis (macrohaematuria) • Chronic glomerulonephritis (more pronounced during aggravation) • Renal infarction (macrohaematuria) • Hypernephroma (periodic macro- and microhaematuria) • Renal tuberculosis (constant microhaematuria) • Congestive kidney (congestive microhaematuria) **N.B.** In the presence of glomerular haematuria, the urine usually contains much protein – so-called protein-erythrocyte dissociation	Unaltered erythrocytes observes more frequently inn urinary tract diseases: • Stones in the pelves, urinary bladder, ureters • Acute cystitis • Malignant tumors • Tuberculosis of urinary bladder or pelves • Hypertrophy of prostate

It is important diagnostically to determine location of bleeding source. A **three-glasses test** is used for this purpose. Patient urinates in three containers (Fig. 1).

Macrohaematuria in the first portion suggests bleeding from urethra (Fig. 1.A), in all three portions – from kidneys or ureters (Fig.1.B), and inn last portion – from urine bladder (Fig.1.C).

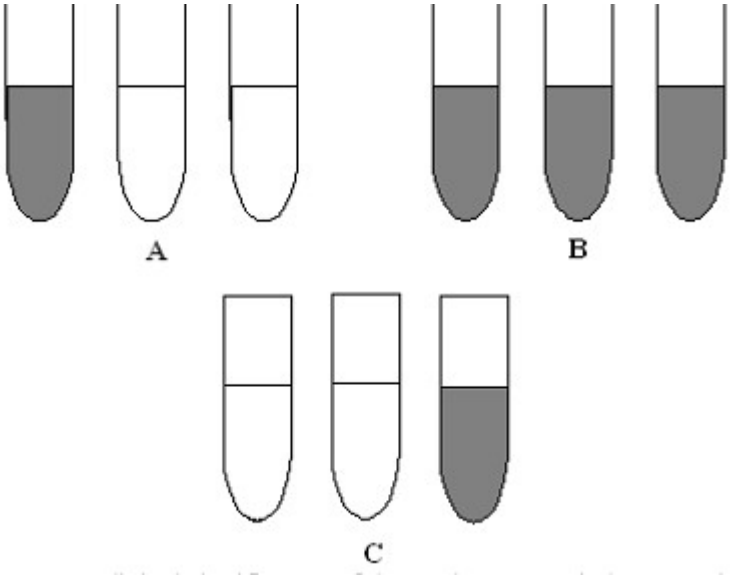

Fig. 1. Clinical significance of three-glasses test in haematuria

Leucocytes are observed mainly in a form of neutrophils, and sometimes eosinophils and lymphocytes are present. Urine of healthy individuals contains small amount of leucocytes (1-2 in vision area). *Leucocyturia* is defined as elevated amount (from 5-6 to 20 cells in vision area) of leucocytes in the urine. *Pyuria* is said to be present when amount of leucocytes increases to 60-100 cells in vision field, and they are seen macroscopically.

Epithelium cells

Tubular (renal) epithelium cells are absent normally in the urine. Their presence indicates acute or chronic affection of the kidneys. They can also be detected in fever, toxicities, and in infectious diseases.

Transitional epithelium cells presence in the urine suggests inflammatory processes in the pelves or bladder.

Squamous epithelium cells originate from genitalia and urethra, and diagnostic their significance is low.

Cylinders (casts). These are cylindrical bodies formed in the lumen of the distal tubule, particularly the collecting tubule. Casts are protein copies of tubules. Appearance of cylinders in urine sediment is called cylinduria - the sign of organic renal diseases.

Hyaline casts are occasionally seen in the urine of normal people, particularly when it is concentrated, or after exercise. Hyaline casts appear in the urine during secondary proteinuria: febrile, congestive, orthostatic, toxic, and after administration of loop diuretics. Constant hyaline casts presence suggests proteinuria of renal genesis: glomerulonephritis, pyelonephritis, and nephropathy.

Granular casts occur in much the same situations as hyaline casts and have similar significance. They are found in the urine of normal subjects after exercise. They appear in many types of renal disease but are particularly characteristic of chronic proliferative or membranous glomerulonephritis, diabetic nephropathy, and amyloidosis.

Waxy casts presence in the urine indicates chronic diseases of the kidneys.

Erythrocytes (unaltered) casts are pathognomic of renal bleeding: nephrolithiasis, tuberculosis and tumor of the kidneys; acute process in the kidneys: acute glomerulonephritis.

Erythrocytes (altered) casts are seen in chronic glomerulonephritis.

Leucocytes casts may appear in considerable numbers during an episode of acute pyelonephritis; a few may be found in the urine in chronic pyelonephritis.

Nechiporenko's method allows counting formed elements in 1 ml of urine, normally:

- Leucocytes – to 4000;
- Erythrocytes – to 1000;
- Casts – to 200.

Crystals. Cystine crystals may be found in freshly passed urine bur are found more consistently if a concentrated sample is acidified and cooled in a refrigerator, their presence is diagnostic of *cystinuria*. Oxalate crystals are common in urine from normal individuals when it has stood for an hour or two. When present in freshly passed urine, in large numbers or aggregates, they may indicate an increased liability to form oxalate stones, but firm conclusions can only be drawn if the urine is kept at 37˚C until examined on a warm-stage microscope.

Mucus. The normal urine practically contains no mucus. Commonly mucus appears in diseases of the urinary tract: urethritis, prostatitis, cystitis, and in stones presence.

It must be emphasized that although urinalysis and microscopy yield valuable information, it is possible for significant renal disease to be present without anything abnormal being detected in the urine.

Bacterioscopic study

Bacteriuria is defined as presence of bacteria in the urine. In quantity not more than 50 000 in 1 ml they may occur in the urine of healthy person. In the presence of bacteriuria, it is important to determine its degree and microorganism sensitivity to various antibiotics.

Tests

1. The earliest complaint of patient with Acute Glomerulonephritis is following:
A. Increasing amount of urine
B. Dark (tea-colored), scanty urine
C. Cloudy urine
D. Straw colored urine
E. Beer-colored urine

2. A 35-year-old man presents with acute onset of fever, shortness of breath and mild oedema of the legs. A 24 hour urine specimen is significant for microscopic hematuria and 1.2g of protein. What is the most likely diagnosis?
A. Nephrotic syndrome
B. Chronic glomerulonephritis

C. Acute glomerulonephritis
D. Chronic heart failure
E. Acute pyelonephritis

3. 17 year old patient was admitted to the hospital because a fever of 40.6º C and shaking chills for the previous day. On physical examination, he had mild right costovertebral angle tenderness. Urinalysis: urine is cloudy; 100 WBC/hpf, 5-10 RBC/hpf, many WBC's casts and occasional transitional cells were found. What is the suspected diagnosis?
A. Acute glomerulonephritis
B. Acute pyelonephritis
C. Chronic glomerulonephritis
D. Kidney stone disease
E. Chronic pyelonephritis

4. A 25-year-old female with 5-years history of urine abnormalities complains of headache, light back pain. Her condition was getting worse 2 weeks ago after cold. Phys exam revealed face edema, BP – 150/95 mm Hg. Urinalysis: protein 0.93 g/24hr, 4-6 RBCs/hpf, gyaline casts – 3-4/hpf, 1-2 granular casts/hpf. What the most possible diagnosis?
A. Chronic glomerulonephritis
B. Chronic pyelonephritis
C. Acute glomerulonephritis
D. Acute pyelonephritis
E. Kidney stone disease

5. A 19-year-old patient with history of a preciding infection (tonsillitis) several days ago presents with dark colored urine. There is puffiness of eyelids, especially in morning and brestlessness. Microscopic hematuria and 2.3g of protein in urine were found. What is the most likely diagnosis?
A. Acute glomerulonephritis
B. Acute pyelonephritis
C. Chronic glomerulonephritis
D. Kidney stone disease
E. Chronic pyelonephritis

6. A 18-year-old male consults his family physician. He has no significant past medical history except periorbital edema last 3 years. Routine laboratory studies revealed proteinuria 0.93 g/24hr, 15-25 RBCs/HPH, 1-2 WBCs/HPF, 2-4 gyaline casts/HPF, 2-3 granular casts/HPF. Phys exam: BP – 140/90 mm Hg. What the most likely diagnosis?
A. Acute pyelonephritis
B. Acute glomerulonephritis
C. Chronic glomerulonephritis, nephritic syndrome
D. Kidney stone disease
E. Chronic pyelonephritis

7. A 45 year old male came to his doctor after spending a

second sleepless night with excruciating lower abdominal pain. The pain seemed to come in waves and was unrelieved by aspirin, tylenol, or lying or standing in any position. He had not experienced any similar pain before. Urinalysis revealed dark yellow, cloudy urine with no protein; 2-5 WBC/hpf, >100 RBC/hpf, no casts and occasional squamous epithelial cells. What diagnosis do you suspect?

A. Acute pyelonephritis
B. Acute glomerulonephritis
C. Chronic glomerulonephritis
D. Kidney stone disease
E. Chronic pyelonephritis

8. A 16-year-old girl brought to family physician for increasing facial and peripheral edema of eight days duration. Urine - 4.2 g protein/24 hrs, hyaline casts, oval fat bodies. What diagnosis do you suspect?

A. Acute pyelonephritis
B. Acute glomerulonephritis, nephrotic syndrome
C. Chronic glomerulonephritis
D. Kidney stone disease
E. Chronic pyelonephritis

9. A 17 year-old girl is admitted to the hospital because of fever, polyuria, nausea and vomiting. Physical exam reveals temperature of 38.9ºC, left flank tenderness. Initial laboratory work shows WBC count 17,800 (N-3,800-10,600) with 85 % neutrophils and 8% bands. Urinalysis: protein - 0.033g/l, WBC – ½ HPF, rare WBC casts, numerous bacteria, and 10-15 RBC per HPF with no RBC casts. What is this patient's diagnosis?

A. Nephritic syndrome
B. Acute renal failure
C. Lithiasis
D. Acute urinary tract infection
E. Acute glomerulonephritis

10. A 52-year-old man was admitted to the hospital. Over the last few months he noted legs edema, face edema, severe weakness, and difficulties of breathing. Phys exam revealed anasarca, BP - 200/110 mmHg. Labs: blood glucose – 12 mmol/l, Urinalysis: Sp,gr – 1.035, protein – 1.3 g/24hr, , glucose – 0.5%. What the most possible reasons for urinalysis findings?

A. Kidney stone disease
B. Acute urinary tract infection
C. Diabetes mellitus, diabetic nephropathy
D. Chronic glomerulonephritis
E. Acute glomerulonephritis

Keys: 1 B, 2 C, 3 B, 4 A, 5 A, 6 C, 7 D, 8 B, 9 D, 10 C

Methodical instructions

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