Physical examination of urine

a) Naked-eye appearances

Urochrome is the chief pigment and is responsible for the amber colour of normal urine. In addition, normal urine contains traces of urobilin and other pigments. Urobilinogen is colourless, contained also in traces. Irrespective of pathological conditions, urine may be pale owing to intake of large volumes of fluid or deeper in colour (more orange) owing to copious sweating or to reduction of fluid intake (= "concentrated"). Substances which may make urine depart from normal amber:

* red - blood, hemoglobin, myoglobin, beets
* port-wine - porphyrin
* brownish-black - melanin (oxidation of melanogen), alkaptonuria (oxidation of homogentisic acid)
* brown - bilirubin, methemoglobin
* orange - small amount of bilirubin
* greenish - biliverdin (oxidation product of bilirubin)
* deep yellow - riboflavin, tetracycline antibiotics, certain chemotherapeutic
* white - chyluria

Under naked-eye appearance will be noted also whether urine is clear or cloudy, whether there is any deposit visible (see the examination of urinary sediments). As a rule phosphates are deposited when the urine is alkaline and urates or uric acid when it is acid. Brownish urates may be then dissolved in KOH, phosphates in acetic acid. The deposits mentioned in a cooled specimen are in most cases of no significance. On the other hand turbidity caused by the massive occurrence of white blood cells microorganisms is always pathological.

b) Odour

There may be a characteristic odour, as, for example, of ammonia due to decomposition by bacteria (increased pH), or unpleasant odour of phenylacetic acid in phenylketonuria.

c) Volume

An average urine output ranges from 1 - 1.5 l/day. *Oliguria* is said to be present when the urine amount is less than 400 ml/day, *anuria* less than about 100 ml/day. When the urine output exceeds 2.5 l/day, *polyuria* is present.

d) Density

The specific gravity may be taken with an urinometer. It ranges from about 1.003 to 1.035.

Procedure:

Collect urine into a clean vessel provided. Record colour and odour. Measure the density (specific gravity) with urinometer.
Basic chemical examination of urine
(proteins, glucose, ketone bodies, blood and hemoglobin, bilirubin, urobilinogen)

To see the positive reaction, please use as "a sample being tested" the imitation of urine positive for the presence of the analyte tested (container labeled with the name of the analyte). Of course, you may try to do the tests with a sample of your own urine. However, being healthy, results must be negative.

1. Proteins
   
   a) Sulfosalicylic acid test

   Place about 1 ml of the sample being tested into a test tube and add 5-10 drops of 20% sulfosalicylic acid. A white precipitate is produced if protein is present. This is a very sensitive test.

   b) Heller's test

   Place carefully about 1 ml of concentrated nitric acid (warning: corrosive !) in a test tube. Incline the tube and slowly pour down the side of the tube in a manner to produce a stratification (two separated layers) about 1 ml of the sample being tested. A white ring appears between the two layers if the test is positive.

   c) Heat coagulation

   Place about 1 ml of the sample being tested into a test tube, add about 0.2 ml of acetate buffer (pH=4.6) and heat to boiling. If protein is present, white turbidity appears.

2. Glucose
   
   a) Fehlings's test

   Take a clean test tube and prepare Fehling's reagent by mixing equal volumes (about 1 ml) of Fehling I (copper(II) sulfate) and Fehling II (NaOH, NaK – tartarate). The reagent prepared is dark blue in colour, without any precipitate inside. Take another test tube and put there about 1 ml of the sample being tested. Add equal volume of Fehling's reagent prepared in the previous step. Heat the content of the test tube to boiling. If the test is positive, reddish brown (orange, olive-green) precipitate is formed.

   b) Benedict's test

   Place about 1 ml of Benedict's reagent in a test tube. Add 4-5 drops of the sample being tested and heat the content of the test tube to boiling. If the test is positive, reddish brown (orange, olive-green) precipitate is formed. *(In principle, Benedict's reagent is only a modification of Fehling's reagent.)*

   c) Nylander's test

   Place about 1 ml of the sample being tested into a test tube, add about 1 ml of Nylander's reagent and heat the content of the test tube to boiling. If the test is positive, the solution turns grayish-yellow due to formation of black precipitate of metallic bismuth.
3. Ketone bodies

a) Lestradet's test

Take a small round filter paper and place it unfolded on the white tile. Use a little spoon (it is inside the plastic box with the reagent) to put Lestradet's reagent onto the center of filter paper. Moisten the reagent on the filter paper with a drop of the sample being tested. If purple colour develops within 1 minute, the test is positive.

b) Legal's test

Take a clean test tube and dissolve few grains of solid sodium nitroprusside in about 1 ml of water. Take another test tube and put there about 5 ml of the sample being tested, add 5 drops of sodium nitroprusside solution prepared in the previous step and 5 drops of 10% NaOH. Red colour appears due to the presence of creatinine. Add few drops of concentrated acetic acid. If ketone bodies are present, the coloration turns to deeper colour.

4. Blood and hemoglobin

Heitz - Boyer's test

In a test tube combine about 1 ml of the sample being tested with equal volume of the Heitz-Boyer reagent. Carefully overlay with hydrogen peroxide. In the presence of hemoglobin (blood) a red-violet ring appears at the interface of two layers.

5. Bilirubin

a) Naumann's test

In a test tube, mix about 5 ml of the sample being tested with talc powder. Prepare what you need for filtration (little funnel, filtrate paper) and filter the mixture to separate talc with bilirubin adsorbed. After the filtration, put a drop of Fouchet's reagent (a solution of FeCl₃ and trichloroacetic acid) on the talc on filtration paper. A blue colour indicates that bilirubin is present. This test is more sensitive than the other tests.

b) Hamarsten's test

Place carefully about 0.5 ml of the mixture of acids (HCl and HNO₃ - warning: corrosive !) in a test tube. Add about 2 ml of ethanol and few drops of the sample being tested. In the presence of bilirubin, green coloration appears.

c) Gmelin's test

Place carefully about 1 ml of concentrated nitric acid (warning: corrosive !) in a test tube. Incline the tube and slowly pour down the side of the tube in a manner to produce a stratification (two separated layers) about 1 ml of the sample being tested. A green ring appears between the two layers if the test is positive.  

(Very same procedure as described for Heller's test; protein – white ring, bilirubin – green ring.)

d) Rosin's test

Place about 1 ml of the sample being tested into a test tube. Carefully overlay with alcoholic solution of iodine. A green ring at the interface of two layers indicates the presence of bilirubin.
6. Urobilinogen

_Ehrlich's aldehyde test_

Place about 1 ml of the sample being tested into a test tube. Add few drops of Ehrlich's aldehyde reagent. A red colour suggests that "Ehrlich positive substances" are present. This is a very sensitive test.

**Diagnostic strips**

The principal benefits of the diagnostic strips are simplicity and speed of the testing procedure (results obtained within 1-2 minutes) as well as a high level of diagnostic specificity and sensitivity. The strips are to be used mainly by general practitioners and specialized physicians.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Principle of the test</th>
<th>Colour scale</th>
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<tbody>
<tr>
<td>Haemoglobin</td>
<td>oxidation of chromogene by organic hydroperoxide in the presence of the haemoglobin</td>
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<tr>
<td>Erythrocytes</td>
<td></td>
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<tr>
<td>Ketones</td>
<td>sodium nitropruside in alkaline buffer (Legal's test)</td>
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<tr>
<td>Bilirubin</td>
<td>reaction of diazonium salt in acidic surroundings</td>
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<tr>
<td>Urobilinogen</td>
<td>reaction of diazonium salt in acidic surroundings</td>
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<tr>
<td>Glucose</td>
<td>enzymatic reaction - glucose oxidase, peroxidase, chromogene</td>
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<tr>
<td>Protein</td>
<td>protein error of pH indicator - mixed acido-basic indicator changes colour in the presence of proteins</td>
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<tr>
<td>pH</td>
<td>mixed acido-basic indicator</td>
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