

# Urine Analyzes

---

## Urine pH and urinal sediment

### Urine pH

#### Method description

It is necessary to determine pH of the freshly taken urine, as old and not well preserved urine is usually contaminated by bacteria. Urine pH of healthy patient is usually influenced by diet and possible medication and can vary from pH 4,7 to 8,0. Protein rich diet moves pH to acidic end of pH scale (acidurie), vegetarian and vegan type of diet moves pH to alkaline (alkaliurie). Standard diet is characterised by pH of about 6. Permanent deviations of the urine pH can participate in formation of urinary stones. In acidic pH the concrements can be usually formed by urates, oxalates and even by cystine. In alkaline pH, the phosphate and carbonate stones can be formed. The therapy is based on urine pH change (acidification by  $\text{NH}_4\text{Cl}$ ) or alkalization.

#### Procedure

Using the pH paper test strip, determine the pH of given sample.

#### Observation

## Urinal sediment

### Method description

Important additional part of chemical urine analysis is microscopic observation of the urinary sediment. The fresh urine has to be used, as longer delay can cause the formation of newly developed bacterial flora and some parts of sediment can change (dissolved, crystallize etc.). The sediment can be used native or coloured.

The parts of sediment:

#### 1. Non-organic – usually crystallized compounds :

In acidic urine: uric acid, calcium oxalate, hippuronic acid (jehlice, rombická prizmata), calcium sulphate, cystin, tyroxin, lucin, bilirubin

V alkaline urine: ammonium urate, magnesium ammonium phosphate, calcium phosphate, calcium carbonate, indigo, cholesterol, medications etc.

#### 2. Organic:

Epithelia: large polygonal, cylindrical, small round or polygonal

Leukocytes (leukocytourie, pyurie)

Erythrocytes (erythrocytourie, haematurie)

Cylinders: homogenous (hyaline and wax), epithelial, leukocytal, erythrocytal, granulated, mixed and pseudocylinders

Fungi etc.

#### 3. Accidental contaminants:

Fibres, hair, sperms, fungi etc.

### Procedure

5 ml of urine spin in centrifuge 10 minutes at 2000 rpm. Using sediment, prepare the microscopic sample.

### Observation

## Qualitative tests of pathological compounds in urine

### Protein test

#### Method description

Test-tube protein tests are based on denaturation and coagulation reactions caused by boiling of them or by strong acids. The use of fresh filtered urine is necessary. Volumes are only approximate.

#### Procedure

**Reaction with sulphosalicylic acid.** 2 ml of urine mix thoroughly with 0.5 ml of sulphosalicylic acid. Opalescent coagulate is prove of presence.

#### Observation

## Haemoglobin or blood presence test

### Method description

The redox reactions are used for blood and haemoglobin test. The reaction itself is based on catalytical properties of iron, causing the substrate to be oxidised by  $\text{H}_2\text{O}_2$  ("pseudoperoxidase" reaction).

### Procedure

Dissolve several crystals of o-tolidine in 1 ml of methanol, acidify by acetic acid, and add 2 ml of  $\text{H}_2\text{O}_2$  (the solution must not turn blue). Then add 1 ml of urine. Blue-green colour indicates the presence of haemoglobin or blood in urine.

### Observation

## Qualitative saccharide test

### Method description

These tests are based on reduction properties of glucose (and galactose). As proteins can influence these tests, these should be removed by boiling the sample followed by filtering. The glucose testing paper strips are based on glucose enzymatic reaction.

### Procedure

Using glucose testing paper strip (GlucoPhan), determine the urine glucose concentration/presence .

### Observation

## **Ketones (acetone) test**

### **Method description**

Acetone reacts with sodium nitropruside in alkaline environment coloured complex. However, this complex is formed in the presence of creatinine. The acetone formed one can be distinguished via acetic acid acidification.

### **Procedure**

Put a small amount of powdered Lestradet's reagent on filtration paper, and wet with a small drop of urine. In presence of ketones, the violet colour will appear in 1 minute.

### **Observation**

## Bile acids test

### Method description

Group of bile acids in urine are: conjugated bilirubin, stercobilinogen urobilinogen and their oxidative products, such as stercobilin and urobilin.

**Ehrlich's test:** Urobilinogen and stercobilinogen form with Ehrlich's aldehyde reagent red colour in 5 minutes. Reaction is not specific and conjugated bilirubin can interfere (also indol, skatol and porphobilinogen). Ehrlich's test has to be carried out with fresh urine, as oxidative products like urobilin and stercobilin can lead to wrong results.

### Procedure

In test-tube mix approximately 2 ml of cloudy urine with 0.5 ml of Ehrlich's reagent. Red colour should appear in 5 minute. Green colour reveals the presence of conjugated bilirubin, together with nitrites. Orange-yellow colour appears in presence of sulphonamides, PAS (p-aminosalicylic acid) or PAH.

### Observation