Date ..... Name ..... Group .....

Instructions for the practical lesson on biochemistry

Topic: **Examination of urine**

Task 1: Qualitative estimation of pathological components of urine

Perform each of the following test-tube reactions with a sample of physiological urine, a sample containing the relevant pathological component, as well as with all the unknown samples of urine.

A. Protein in urine

Reagents:

1. Sulfosalicylic acid dihydrate 200 g/l
2. Sample of urine with protein
3. Sample of physiological urine
4. Unknown samples of urine 1-4

Procedure:

- **Test with sulfosalicylic acid:** to 1-2 ml of sample add a few drops of aqueous sulfosalicylic acid. Appearance of turbidity or even precipitate indicates presence of protein. The change is best evaluated against a black background or a page with printed text.

Semi quantitative evaluation of the sulfosalicylic acid test:

Appearance:	*Evaluation*	*Approximate protein concentration:*
Opalescence	Traces	0.05 – 0.1 g/l
Slight turbidity (transparent, a text behind the tube is legible)	+	0.1 – 0.2 g/l
Opaque turbidity (not transparent, without flakes)	++	0.5 – 1.0 g/l
Milky turbidity with flakes	+++	2.0 – 5.0 g/l
Cheese-like precipitate	++++	above 5.0 g/l

B. Blood and blood pigment in urine

Reagents:

1. o-tolidine (3,3'-dimethylbenzidine)
2. Heitz-Boyer's reagent: colorless reduced phenolphthalein in alkaline medium, stored with several granules of zinc 

3. Acetic acid concentrated 
4. Hydrogen peroxide 30 g/l 
5. Ethanol 
6. Sample of urine with blood
7. Sample of physiological urine
8. Unknown samples of urine 1-4

Procedure:

- **"Benzidine" Test:**

Dissolve a few grains of o-tolidine in about 2 ml of ethanol and acidify with concentrated acetic acid (few drops). Add about 2 ml of hydrogen peroxide (the solution must not turn blue at this stage) and combine with 1-2 ml of the urine sample. If the solution turns blue or blue-green the test is positive.

- **Heitz-Boyer's Test:**

In a test tube combine about 1 ml of urine with equal volume of the Heitz-Boyer reagent. Carefully overlay with hydrogen peroxide. In the presence of hemoglobin a red-violet ring appears at the interface.

C. Sugar in urine

Reagents:

1. Fehling's solution I: Copper(II) sulfate cryst. 70 g/l 
2. Fehling's solution II: Sodium hydroxide 250 g/l 
   Potassium-sodium tartrate cryst. 350 g/l
3. Sample of urine with glucose
4. Sample of physiological urine
5. Unknown samples of urine 1-4

Procedure:

- **Fehling's test**

Prepare a fresh Fehling's reagent before testing for the presence of glucose. Mix the Fehling's solution I (copper sulfate) and II (NaK-tartrate with NaOH) in ratio 1:1. Heat a portion of about 1 ml of the Fehling's reagent to boiling – it must not change color. By this way presence of reducing agents is excluded.

Mix about 1 ml of urine sample with a similar amount of cold (new portion) Fehling's reagent in a test tube. Boil in water bath. If the glucose or another reducing compound is present, a green-yellow, yellow, or even a brick-red precipitate develops. The color depends on the amount of glucose in the urine sample (turbidity with greenish tint – about 8-15 mmol/l glucose, green ppt. – about 25 mmol/l, greenish-brown ppt. – about 50 mmol/l, brownish-red ppt – about 100 mmol/l, red ppt – over 150 mmol/l glucose).

D. Ketone bodies:

Reagents:

1. Sodium nitroprusside cryst. 
2. Sodium hydroxide 100 g/l 
3. Glacial (concentrated) acetic acid 
4. Lestradet's reagent:  ammonium sulfate 20 g, sodium carbonate anhydrous 20 g, sodium nitroprusside 0.2 – 1 g.
5. Sample of urine with ketone bodies
6. Sample of physiological urine
6. Unknown samples of urine 1-4

Procedure:

- **Lestradet's test:**

Place a circle of filter paper onto a watch glass and wet it with distilled water. Put a small amount of Lestradet's reagent (powder) on the filter paper and add 1 – 2 drops of urine. A purple color developing within 1 minute indicates presence of ketone bodies.

- **Legal's nitroprusside test:**

Dissolve few crystals of sodium nitroprusside in a test tube with distilled water.
To about 2 ml of urine add 2 – 3 drops of aqueous solution of sodium nitroprusside and alkalize with 3 drops of NaOH. A red color appears that is caused by creatinine (physiological component of urine). Divide the colored solution into two parts. Add a few drops of glacial acetic acid into one part of solution: if the color changes to yellow it was caused by creatinine. In contrast, in the presence of ketone bodies the red color turns to red-violet upon addition of the acetic acid.

E. Examination of urine with polyfunctional diagnostic strips

Perform the examination with diagnostic strips only with the unknown samples of urine.

Reagents:

1. Polyfunctional strip PHAN (Erba-Lachema Diagnostica s.r.o.)
2. Unknown samples of urine 1-4

Procedure:

Immerse the strip into urine for 2-3 seconds. Wipe edge of the strip against rim of the test tube to remove excess urine. Wait 60 seconds (120 sec. for leukocytes) and compare the test zones on the strip with the color scale on the tube label.

Task 2: Demonstration of semiautomatic reflectance photometer for objective semi-quantitative analysis of urine with diagnostic strips

Reagents and equipment:
1. LAURA Smart reader
2. Diagnostic strips for LAURA Smart
3. Urine samples

Procedure:
This measurement will be demonstrated by your instructor.

Task 3: Measurement of the relative specific gravity of urine with urinometer

Procedure:
A suitable cylinder is filled with urine sample. Urinometer is dipped to the urine and the relative specific gravity of the sample is read from its scale.

This measurement will be demonstrated by your instructor.

Task 4: Examination of urinary sediment in phase contrast

Reagents:
Authentic sample of fresh urine – **infectious material**

Procedure:
A fresh sample of urine has been centrifuged at 400– 600 g for 10 minutes at 10 °C and kept on ice. Majority of supernatant has been removed and the urinary sediment is resuspended in the remaining small volume of supernatant. A microscopic preparation has been made from the concentrated urinary sediment. Your task is to examine this preparation under microscope with phase contrast.

Results and conclusion:
Describe the findings in the preparation of urinary sediment