Comenius University in Bratislava, Faculty of Medicine
Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

LABORATORY PROTOCOL SS08

Determination of calcium in blood serum and urine

Name, group:	Date:

The purpose of the laboratory experiment is to determine total concentration of Ca^{2+} ions in the serum and in the urine (which was gathered for 24 hours and was properly acidified and mixed). We will compare the measured concentrations and evaluate changes in Ca^{2+} concentrations with regard to regulation mechanisms.

Principle:
Glykoxal-bis-(2-hydroxyanil) forms red-colored complex with Ca^{2+} ions in alkaline solution.

Procedure:

	S_1	S_2	U_1	U_2	ref. sample
serum 1	0,5 ml	---	---	---	---
serum 2	---	0,5 ml	---	---	---
urine 1	---	---	0,5 ml	---	---
urine 2	---	---	---	0,5 ml	---
water	---	---	---	---	0,5 ml
reagent mixture	2,0 ml	2,0 ml	2,0 ml	2,0 ml	2,0 ml
We let the samples stand for 10 minutes and measure absorbance at 525 nm.					

Calculation:
Diuresis: patient 1: 1,5 l/24 hours; patient 2: 1,2 l/24 hours

	Patient 1		**Patient 2**	
	S_1	U_1	S_2	U_2
absorbance				
Ca^{2+} (mmol/l)				
Ca^{2+} (mmol/24 hours)	---		---	

Reference values:
in serum: **2,25 – 2,75 mmol/l**
in urine: **2,5 – 7,5 mmol/ 24 hours**

Determination of inorganic phosphate in serum

Principle: Inorganic phosphate reacts with ammonium molybdate in acidic environment to give phosphomolybdate, which then is reduced into blue coloured salts of molybdenium V.

Preparation of serum:0,3 ml of blood serum + 4,7 ml water + 1,0 ml 25% TCA
Precipitated proteins are removed by centrifugation and for determination we use supernatant (containing serum diluted 1:20)

	Serum 1	Serum 2	Blank
Serum 1	1,5	-	-
Serum 2	-	1,5	-
H_2O	-	-	1,5
Ammonium molybdate	0,4	0,4	0,4
$SnCl_2$	0,3	0,3	0,3
Stay 10 minutes in laboratory temperature and measure absorbance at 660 nm			

Reference values:

Serum: **0,72 – 1,37 mmol/l**

Conclusion:

Next week:
- new topic (acid-base balance)