

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

LABORATORY PROTOCOL SS - 8th seminar
Determination of calcium and phosphate

Name, study group:	Date:
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Determination of calcium in blood serum and urine

Principle:

Total concentration of Ca²⁺ ions in the serum and in the urine is determined. Urine is collected for 24 hours and then, it is sufficiently acidified, mixed and measured. The measured results will be assessed according to changes in calcium concentration with respect to regulatory mechanisms.

Glyoxal-bis-(2-hydroxyanil) forms red-coloured complex with Ca²⁺ 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

Let the samples stand for 10 minutes and measure absorbance at 525 nm.

Calculation:

	Patient 1		Patient 2	
	S ₁	U ₁	S ₂	U ₂
absorbance				
Ca ²⁺ (mmol/L)				
Ca ²⁺ (mmol/24 hours)	---		---	

Diuresis: patient 1: 1.5 L/24 hours; patient 2: 1.2 L/24 hours

Reference values:

Calcium in serum: **2.25 – 2.75 mmol/L**

Calcium in urine: **2.5 – 7.5 mmol/ 24 hours**

Determination of inorganic phosphate in serum

Principle:

Phosphomolybdate is formed by the reaction of inorganic phosphate with ammonium molybdate in an acidic environment. Then, the phosphomolybdate is reduced to the blue coloured salts of molybdenum (in oxidative state V).

Preparation of serum:

Precipitation of proteins: 0.3 mL of blood serum + 4.7 mL water + 1.0 mL 25% TCA
Precipitated proteins are removed by centrifugation. Then, supernatant is used for determination of inorganic phosphate (serum is diluted 1:20).

	Serum 1	Serum 2	Blank
serum 1 (1:20)	1.5 mL	-	-
serum 2 (1:20)	-	1.5 mL	-
H ₂ O	-	-	1.5 mL
ammonium molybdate	0.4 mL	0.4 mL	0.4 mL
SnCl ₂	0.3 mL	0.3 mL	0.3 mL
Let the samples stand for 10 minutes and measure absorbance at 660 nm			

Calculation:

	Patient 1	Patient 2
	S ₁	S ₂
absorbance		
phosphate (nmol/1.5 mL)		
correction for dilution		
phosphate (mmol/L)		

Reference values:

Phosphate in serum: **0.72 – 1.37 mmol/L**

Conclusion:

Next week:

Acid-Base balance and pH

(Study material: uploaded to MS Teams MF_Medical Biochemistry-GM/D _2023-2024)