

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

LABORATORY PROTOCOL GM-WS - 7th seminar
Determination of ketone bodies in blood serum

Name, group:	Date:
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Principle:

Ketone bodies - acetoacetate and acetone – form colored complex with nitroprusside in presence of glycine.

sample	S ₁	S ₂	standard	reference
serum 1 (dilution 1:10)	0.5 ml	---	---	---
serum 2 (dilution 1:10)	---	0.5 ml	---	---
standard (0.006 mmol/l)	---	---	0.5 ml	---
water	---	---	---	1.0 ml
phosphate buffer	2.0 ml	2.0 ml	2.0 ml	2.0 ml
glycine	0.5 ml	0.5 ml	0.5 ml	---
nitroprusside	0.5 ml	0.5 ml	0.5 ml	0.5 ml

We let the samples stand for 30 minutes and measure absorbance at 590 nm.

Calculation:

$$c(\text{sample}) = (A_{\text{sample}} / A_{\text{standard}}) \times C_{\text{standard}}$$

Acetoacetate and acetone make up only 1/4 of total amount of ketone bodies in blood serum, 3/4 of total amount is represented by β -hydroxybutyrate.

	Patient 1	Patient 2	
sample	S ₁	S ₂	standard
absorbance			
acetoacetate + acetone (mmol/l)			0.006
correction for dilution			---
total concentration of ketone bodies			---

Reference values: less than 0.25 mmol/l

Conclusion:

Literature for next week:

Lipid metabolism, oxidation and synthesis of fatty acids, synthesis and degradation of triacylglycerols.

- Lippincott's: Chapter 16, Part I - IV;