

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

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LABORATORY PROTOCOL GM-WS - 8<sup>th</sup> seminar  
Determination of unesterified fatty acids in blood serum

Name, group:	Date:
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Principle:

Lipids from bloodserum are extracted by the mixture ethanol/diethyleter (3:1) To 1 ml of serum 9 ml of mixture ethanol/diethyleter is aded and mixed. After centrifugation clean extractwe use for lipid determination.

We determine unesterified fatty acids in three situations:

- a) after carbohydrate meal
- b) fasting
- c) in diabetics

Principle: Unesterified fatty acids are determined by neutralization titration with solution of NaOH and using indicator bromothymol blue (in acidic environment is yellow coloured, in alkaline environment is blue)

Procedure:

Sample	1	2	3
Lipidic extract 1	0,5		
Lipidic extract 2	-	0,5	
Lipidic extract 3	-	-	0,5
Bromothymol. blue	0,1	0,1	0,1
	Titration with solution of NaOH - NaOH we add carefully by drops, with continous mixing - we titrate until change to blue colour which stays minimum 5 seconds - every titration we repeat two times, for calculation we use average from two titrations - amount of used NaOH – 1 drop = 50 µl		

From calibration graph according to used NaOH we read amount of unesterified fatty acids in the sample and calculate concetration of fatty acids in mmol/l.

Physiological values: 0,3 – 1,2 mmol/l

Conclusion: