

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

LABORATORY PROTOCOL SS - 1st seminar
Differential diagnosis of megaloblastic anemia

Name, study group:	Date:
--------------------	-------

The measurement of elevated concentration of methylmalonic acid (MMA) in the blood or urine serves as a sensitive and early indicator of vitamin B₁₂ deficiency. It is one of the possible causes of megaloblastic anemia (the other possible cause is folic acid deficiency). Vitamin B₁₂ is required for two main enzymatic reactions:

- conversion of methylmalonyl-CoA to succinyl-CoA by *methylmalonyl-CoA-mutase*
- synthesis of methionine from homocysteine by *methionine-synthase*

Without sufficient B₁₂ concentration, the methylmalonyl-CoA conversion to succinyl-CoA is decreased. Therefore, the concentration of MMA increases in the serum and urine over the time. On the other hand, in folic acid deficiency there is no elevation of MMA in the serum and urine.

Principle:

Methylmalonic acid (MMA) was separated from the urine using Dowex AG column. A random urine sample (**5 mL**) was applied to a Dowex column and washed by 50 mL of distilled water. MMA was eluated using **20 mL** of 0.1 mol/L HCl, resulting in 20 mL of acidic eluate. MMA then reacts with diazo-reagent forming blue compound.

Procedure:

	U ₁	U ₂	reference sample
acidic eluate 1	1 mL	---	---
acidic eluate 2	---	1 mL	---
0.1 mol/L HCl	---	---	1 mL
acetate buffer	0.5 mL	0.5 mL	0.5 mL
diazo-reagent	0.5 mL	0.5 mL	0.5 mL
Incubate the samples for 5 min. at laboratory temperature.			
3 mol/L NaOH	0.5 mL	0.5 mL	0.5 mL
Mix the samples and measure the absorbance at 620 nm.			

Calculation:

	Patient 1 - U ₁	Patient 2 - U ₂
absorbance		
nmol/1 mL of eluate (from cal. curve)		
nmol/20 mL of eluate (in 5 mL of urine)		
nmol/L of urine		
mmol/L of urine		
mmol per day		

Diuresis: patient 1: 1.0 L/24h
 patient 2: 1.2 L/24h

Reference values:

Excretion of MMA by urine in patients with B₁₂ deficiency is **higher than 0.2 mmol/24 hours**.

Conclusion:

Literature for 1st week:

Lippincott's:

- Chapter 28: Vitamins

Literature for next week:

Lippincott's:

- Chapter 22: part I-V - Purine nucleotide metabolism, hyperuricaemia