

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

LABORATORY PROTOCOL SS01
Differential diagnosis of megaloblastic anemia

Name, group:	Date:
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The measurement of elevated amounts of methylmalonic acid in the blood or urine serves as a sensitive and early indicator of vitamin B₁₂ deficiency which 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*

If there is not enough B₁₂ available, then MMA concentration starts to elevate resulting in an increase of MMA in the blood and urine. This is in contrast with folic acid deficiency, in which there is no elevation of methylmalonic acid in serum and in urine.

Principle:

Methylmalonic acid 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. Methylmalonic acid was eluated using 20 ml of 0.1 mol/l HCl. We obtained 20 ml of acidic eluate. MMA reacts with diazoreagent 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
diazoreagent	0.5 ml	0.5 ml	0.5 ml
We let the samples stand for 5 min at laboratory temperature.			
3 mol/l NaOH	0.5 ml	0.5 ml	0.5 ml
We mix the samples and measure absorbance at 620 nm.			

Calculation:

	Patient 1	Patient 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 produced 1.2 L of urine per day; patient 2 produced 1.0 L of urine per day.

Reference values:

The amount of MMA in urine in patients without B₁₂ deficiency is **less than 0.2 mmol per day**.

Conclusion:

Next week:

- new topic (purine nucleotide metabolism, hyperuricaemia)

Literature: Lippincott's Biochemistry: chapter 28 - Vitamins and chapter 22 - Nucleotide metabolism