

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

LABORATORY PROTOCOL GM-WS - 12th seminar
Determination of creatinine in blood plasma and urine,
determination of glomerular filtration

Name, group:	Date:
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Principle:

Creatinine forms with picric acid in alkaline solution (Jaffe reaction) orange-red complex. Addition of sodium dihydrogenphosphate into the solution with formed color complex causes decoloration of not reacted compounds thus intensifying the coloration of the solution.

Procedure:

	serum (1:10)	urine (1:500)	standard	reference
We pipette into the test tube	1.0 ml of supernatant	1.0 ml of urine	1.0 ml of standard	1.0 ml of water
picric acid	0.2 ml	0.2 ml	0.2 ml	0.2 ml
20 % NaOH	0.4 ml	0.4 ml	0.4 ml	0.4 ml
We let the samples stand for 15 minutes.				
NaH ₂ PO ₄	2.0 ml	2.0 ml	2.0 ml	2.0 ml
We let the samples stand for 5 minutes and measure absorbance at 500 nm.				

Calculation:

Diuresis: 1.0 l of urine / 24 hours

Male patient: age: 50 years; weight: 70 kg.

	serum (S _{crea})	urine (U _{crea})	standard
absorbance			
creatinine (μmol/l)			15
correction for dilution			---

Clearance of creatinine – a measurement of glomerular filtration – in **ml/min** or **ml/s**:

$$Cl_{crea} = (U_{crea} \times V_{urine \text{ per minute or per second}}) / (S_{crea})$$

Approximate determination of glomerular filtration (in **ml/s**) using Cockcroft formula:

$$Cl_{crea} = [(140 - \text{age}) \times \text{weight}] / [48.8 \times S_{crea}] \times F$$

(Age is inserted in the equation in years; weight in kg; S_{crea} in $\mu\text{mol/l}$)
(F = factor of gender; F(male) = 1; F(female) = 0.85)

Reference values:

serum (S_{crea})	50 – 110 $\mu\text{mol/l}$
urine (U_{crea})	7- 20 mmol/24 hours
clearance of creatinine	2 ml/s (1.3 – 2.8 ml/s); 120 ml/min

renal function	clearance of creatinine (ml/s)	clearance of creatinine (ml/min)
normal function	1.3 – 2.8	80 – 168
light disorder	1.0 – 1.3	60 – 80
mild disorder	0.67 – 1.0	40 – 60
severe malfunction	0.33 – 0.67	20 – 40
renal failure	< 0.33	< 20

Conclusion:

Literature for next week: topics for credit test