

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

LABORATORY PROTOCOL GM-SS - 11th seminar

Determination of total and conjugated bilirubin in blood serum

Name, study group:	Date:
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Principle:

Bilirubin with diazotized sulfanilic acid in an alkaline environment gives an azo dye (van den Bergh reaction). According to the speed of the reaction with the diazotizing agent, bilirubin is divided into directly reacting (conjugated) and indirectly reacting (free, unconjugated). The addition of caffeine accelerates the diazotization of unconjugated bilirubin and makes it possible to determine the concentration of both forms. Diazotization of conjugated bilirubin takes place in 5 minutes and the colored product is measured immediately.

Procedure:

Total bilirubin	total bilirubin 1	total bilirubin 2	reference sample
reagent	0.2 mL	0.2 mL	0.2 mL
NaNO ₂	1 drop	1 drop	1 drop
accelerator	1.0 mL	1.0 mL	1.0 mL
serum 1	0.2 mL	---	---
serum 2	---	0.2 mL	---
physiological solution	---	---	0.2 mL
Mix the samples and let them stand for 10 minutes.			
Fehling II	1.0 mL	1.0 mL	1.0 mL
Mix the samples, let them stand for 10 minutes and measure absorbance at 578 nm.			

Conjugated bilirubin	conj. bilirubin 1	conj. bilirubin 2	reference sample
reagent	0.4 mL	0.4 mL	0.4 mL
NaNO ₂	2 drops	2 drops	2 drops
serum 1	0.4 mL	---	---
serum 2	---	0.4 mL	---
physiological solution	2.0 mL	2.0 mL	2.0 mL + 0.4 mL
Mix the samples, let them stand for 5 minutes and measure the absorbance at 546 nm.			

Calculation:

Patient 1			
	total bilirubin	conjugated bilirubin	non-conjugated bilirubin
absorbance			---
concentration (µmol/L)			

Patient 2			
	total bilirubin	conjugated bilirubin	non-conjugated bilirubin
absorbance			---
concentration (µmol/L)			

Reference values:

		total bilirubin	conjugated bilirubin
Newborns	up to 24 hours	up to 80 µmol/L	---
	24 – 48 hours	up to 150 µmol/L	---
	3 – 5 days	up to 200 µmol/L	---
Adults		up to 24 µmol/L	up to 5 µmol/L

Conclusion:

Next week:

- Enzymes of blood plasma and clinical meaning