

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

LABORATORY PROTOCOL SS11

Determination of total and conjugated bilirubin in blood serum

Name, group:	Date:
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Principle:

Bilirubin reacts with diazotized sulphanilic acid to form colored azodipyrrols concentration of which is measured using spectrophotometry. Conjugated bilirubin (water-soluble) reacts rapidly, non-conjugated bilirubin (not water-soluble) requires accelerator for its reaction.

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
We mix the samples and let them stand for 10 minutes.			
Fehling II	1,0 ml	1,0 ml	1,0 ml
We 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
We mix the samples, let them stand for 5 minutes and measure 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:

- new topic (enzymes of blood plasma and clinical meaning)