# Enzymatic colorimetric determination of Total Bile Acids in serum or plasma

## **CLINICAL SIGNIFICANCE**

Total bile acids are metabolized in the liver, so they serve as an indicator for normal liver function. In a healthy organism the bile acids are reabsorbed by the liver and intestines or eliminated through the

faeces. Increased blood concentration levels of bile acids can occur in the presence of acute hepatitis, chronic hepatitis, hepatic sclerosis, liver cancer, Wilson's disease, infectious mononucleosis and undergoing drugs that prevent or slow liver function including cyclosporine , isoniazid, methotrexate, rifampicin, fusidic acid.

#### TEST SUMMARY

In the presence of Thio-NAD, the enzyme 3-αhydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-keto steroids and Thio-NADH.

The reaction is reversibile and 3- $\alpha$ -HSD can convert 3-keto steroids and Thio-NADH to Bile Acids and Thio-NAD.

In the presence of excess NADH, the enzymatic cycling occours efficiently and the rate of formation of Thio-NADH is mdetermined by measuring specific change of absorbance at 405 nm.



## SAMPLES

Fresh serum or plasma with EDTA. Bile Acids concentration is increased after meals,

hence sample should be collected under fasting conditions.

Serum or plasma samples are stable for 1 week at 4°C or for 3 months at -20°C.

## REAGENTS

Reagent 1: Good's Buffer 10 mM; Thionad 2 g/l.

Reagent 2:	Good's Buller 50 milli, Thenanolamyne 30
	ml/l, NADH 0.3 mM, 3-α HSD > 1 KU/l.

Standard: Bile acids soltution 50  $\mu mol/l;$  stabilizers and preservatives.

#### MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Distilled water.

## PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

## REAGENTS PREPARATION

Reagents are supplied in liquid form ready to use. Reagents are stored at 2-8°C until expiration date on label away from light source.

#### PROCEDURE

Kind of analysis:	Kinetics
Reading time:	6, 7 minutes
Wavelength:	405/600 nm
Temperature:	37°C
Zero:	Distilled Water

 Reagents
 Standard
 Sample

 Reagent 1
 270 μl
 270 μl

 Sample
 -- 4 μl

 Standard
 4 μl
 -- 

 Mix and incubate at 37°C for 5 minutes then add:
 Reagent 2
 90 μl
 90 μl

Mix and incubate at 37°C for 60 seconds, read absorbance (A1) and exactly after 60 seconds from first reading read absorbance (A2).

## CALCULATION

Calcolate  $\Delta A/min. = A2 - A1$ 

Bile Acids (µmol/l) = ( $\Delta A$  sample/ $\Delta A$  standard) x 50

#### EXPECTED VALUES

0 - 10 µmol/l

Every laboratory should establish own reference intervals in accordance with own population.

## CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose on request are available the following control solutions.

CC02530 2 x 2 ml BILE ACIDS-Control Set (2 levels)

#### NOTE

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
  - Only for IVD use.

## TEST PERFORMANCE

Precision			
Intra-assay (n = 4)	Mean (µmol/l)	SD (µmol/l)	CV%
Sample 1	7.93	0.31	3.9
Sample 2	12.50	0.21	1.7
Sample 3	23.48	0.30	1.3
Sample 4	52.25	0.63	1.2
Sample 5	98.18	0.62	0.6

Inter-assay (n = 4)	Mean (µmol/l)	SD (µmol/l)	CV%
Sample 1	8.12	0.24	2.9
Sample 2	12.18	0.40	3.3
Sample 3	23.00	0.61	2.6
Sample 4	51.92	0.69	1.5
Sample 5	97.36	1.70	1.8

## Sensitivity/limit of detection

Method is able to discriminate up to 1 µmol/l.

## Linearity

Method is linear up to 180 µmol/l.

#### Methods comparison

A comparison with a commercial available product gave the following result r = 0.99.

#### Interferences

No interferences were obse	erve in presence of:
Bilirubin	≤ 50 mg/dl
Hemoglobin	≤ 50 mg/dl
Triglycerides	≤ 350 mg/dl
Ascorbic Acid	≤ 50 mg/dl

#### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

TOTAL BILE ACIDS

## PACKAGING

CODE CC02500	(110 TESTS)	
Reagent 1	2 x 15 ml	(liquid)
Reagent 2	1 x 10 ml	(liquid)
Standard	1 x 2 ml	(liquid)

#### REFERENCES

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#### SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
X	Storage temperature interval
$\Box$	Expiration date
$\triangle$	Warning, read enclosed documents
ī	Read the directions
\$	Biological risk

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