TOTAL BILE ACIDS

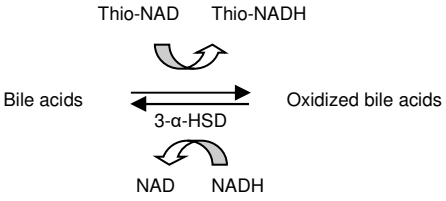
Enzymatic colorimetric determination of Total Bile Acids in serum or plasma

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-α-hydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-keto steroids and Thio-NADH.
The reaction is reversibile and 3-α-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 or Lithium heparin.
Bile Acids concentration is increased after meals, hence sample should be collected under fasting conditions.
(This does not apply to women with intrahepatic cholestasis of pregnancy who will need peak bile acid testing and samples should therefore be taken post-prandially).
Serum or plasma samples are stable for 1 week at 4°C or for 3 months at -20°C.
Specimens from patients, who are on Ursodeoxycholic Acid (UDCA) treatment, are not suitable for use with this Assay.

REAGENTS

Reagent 1: Thio-NAD > 0.1 mM in Buffer Solution.

Reagent 2: NADH > 0.1 mM, 3-α HSD > 2 KU/l in Buffer Solution

Standard: Cholic Acid solution 50 µmol/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.
The intrinsic yellow to yellow-brown colour of the Reagent does not interfere with the test.
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 Sample Standard	270 µl --- 4 µl	270 µl 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

Calculate i ΔA/min. = A2 – A1

Bile Acids (µmol/L) = (ΔA sample/ΔA 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

- As with any diagnostic procedure, if the results are inconsistent with the clinical presentation, the physician should evaluate the data obtained using this test in light of other clinical information.
- Only for IVD use.

PRESTAZIONI DEL TEST

Precisione

Intra-assay (n = 20)	Mean (µmol/L)	SD (µmol/L)	CV%
Sample 1	7.93	0.31	3.9
Sample 2	23.5	0.30	1.3

Inter-assay (n = 20)	Mean (µmol/L)	SD (µmol/L)	CV%
Sample 1	8.12	0.24	2.9
Sample 2	23.00	0.61	2.6

Sensitivity/limit of detection
Method is able to discriminate up to 1 µmol/L.

Linearity
Method is linear up to 180 µmol/L.
If the value is higher than the linearity limit, dilute the sample with physiological solution and repeat the test, multiplying the result by the dilution factor.

Methods comparison
A comparison with an available commercial method gave the following results in a comparison on 52 serum samples having concentrations in the range 0.47 – 131.25 µmol/L:

TBA LTA = y
TBA competition = x
n = 52

y = 1.1536x – 0.8567 µmol/L r = 0,9918

A comparison between samples tested on both serum and plasma with lithium heparin having concentrations in the range 0.14 – 21.18 µmol/L gave the following results:

Plasma with lithium heparin = y
Serum = x

y = 0.9972x +0.1178 µmol/L r = 0,9805

Interferences
No interferences were observed in presence of:

Bilirubin	≤ 50 mg/dL
Hemoglobin	≤ 500 mg/dL
Triglycerides	≤ 750 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.

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

1. La Russo, N.F. et al., Dynamics of Enterohepatic Circulation of Bile Acids, New Engl J M, 291, 689-692, (1974).
2. Skrede S. et al: Bile acids measured in serum during fasting as a test for liver disease, Clin Chem 24: 1095-1099, 1978.
3. Wu, Alan H. B. Tietz Clinical Giude to Laboratory Tests. 4th ed. St. Louis, MO: Saunders/Elsevier, 2006. 170-171.
4. Ovadia C, Seed P, Sklavounos A, et al. "Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy patient data meta-analyses." The Lancet, Elsevier Inc., 14 Feb 2019, dx.doi.org/10.1016/S0140-6736(18)31877-4.
5. CLSI, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline, H18-A4, Vol.30 No. 10.

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SYMBOLS

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

NOTE: CHANGES HIGHLIGHTED WITH A GRAY BACKGROUND