SPINREACT

CE TBA

Total Bile Acids

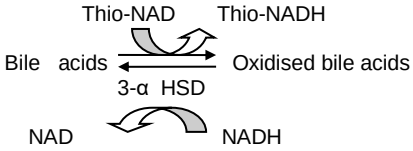
Enzymatic - Colorimetric

Quantitative determination of Total Bile Acids
IVD

Store at 2-8ºC

INTENDED USE
For the quantitative determination *in vitro* of Total Bile Acids in serum and plasma.

PRINCIPLE OF THE METHOD[1,2]
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 reversible and 3-α HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excesss NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405 nm.



CLINICAL SIGNIFICANCE
Fasting serum bile acids can be used in the diagnosis and prognosis of liver disease. Levels rise in many liver diseases, for example hepatitis and liver sclerosis. Abnormal levels in fasting patients or immediately after a meal can be used to detect liver disease and damage, impaired liver function, intestinal dysfunction and perhaps a gall bladder blockage. Bile acid measurement may detect some forms of liver disease earlier than standard liver tests because bile acids levels correspond to liver function, rather than liver damage. In veterinary medicine, bile acid measurement is considered to be a superior indicator of liver disease.

REAGENTS

R 1	Goods buffer Thio-NAD Sodium azide	 1,0 g/L 0,05 % (w/v)
R2	Goods buffer NADH 3-α HSD Sodium azide Stabilizers	 6,0 g/L 12 KU/L 0,05 % (w/v)
OPTIONAL	TBA CAL	REF: 1002290

PRECAUTIONS
R1 and R2 contain Sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.See MSDS for disposal of the product.

PREPARATION
Reagents are ready for use.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Stability: Once opened R1 and R2 are stable for 28 days at 2-8°C.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 405 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES
Serum, EDTA / Lithium heparin plasma. Serum or plasma samples are stable for 1 week at 2-8ºC, or at 3 months at -20ºC.

PROCEDURE
1. Assay conditions:
   Wavelength: 405nm
   Cuvette: . . . . . .1 cm light path
   Constant temperature . . . . . .37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Calibrator	Sample
R1	750 µL	750 µL
R2	250 µL	250 µL
Standard	10 µL	----
Sample	----	10 µL

4. Mix and read the absorbance after 60 s (A_1) and 120 s (A_2).
5. Calculate: $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{(\Delta A)\,Sample}{(\Delta A)\,Calibrator}$$ x Calibrator conc = µmol/L bile acid in the sample

QUALITY CONTROL
Control Sera are recommended to monitor the performance of assay procedures: TBA / CO_2 Control Ref. 1002292.
If control values are found outside the defined range, check the instrument, reagent and calibration for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES
Human Serum (fasting) 0 - 10 µmol/L
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From *detection limit* of 1,47 µmol/L to *linearity limit* of 150 µmol/L.
If the results obtained were greater than linearity limit, dilute the sample 1/5 with NaCl 9 g/L and multiply the result by 5.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (µmol/L)	26,8	42,9	24,9	40,6
SD	0,66	1,08	0,47	0,87
CV (%)	2,48	2,52	1,92	2,14

Sensibilidad analítica: 1 µmol/L= 0,0010784 (A)
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0,987.
Regression equation: y= 0,9755x - 0,17307.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
The following analytes were tested up to the levels indicated and were found not to interfere: Haemoglobin (250 mg/dL), Triglycerides (1000 mg/dL), Intralipid (800 mg/dL) and Bilirubin (85 mg/dL).

NOTES
1. The reagent should not be used if exposed to temperatures above 25ºC for greater than 8 hours, as the accuracy of the assay will be affected.
2. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY
1. Komiyama, Youichi. , Adachi, Tetsuo, Ito, Yoshimasa, Hikano, Kazuyuki. , Sugiura, Mamoru. , Sawaki, Siiunji. Microassay Of Serum Bile Acids By An Enzymatic Cycling Method, Chem Pharm Bull (Toyko) 30: 3796 -3797 (1982).
2. Agape, V., Russo, P., Xaiz, L., Calmi, S., and Grisler, R. Evaluation Of Colorimetric Enzymatic Procedure for Determining The Total Bile Acids In the Blood. Minerva Dietol Gastroenterol. Jul-Sep: 35 (3): 159 – 164 (1989).

PACKAGING

Ref: 1001030	Cont.	R1:1 x 50 mL, R2:1 x 18 mL

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