SPECTRUM

The Creative Approach to Bioscience

Total bile acids (TBA)

Enzymatic recycling method

REF: 305 001 100 test
REF: 305 002 40 test

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	Manufactured by
REF Catalogue Number	(Xi) - Irritant
Consult instructions for use	
Temperature Limitation	

Intended Use

Total bile acids (TBA) diagnostic reagent is used for quantitative in vitro determination of total bile acids in serum or plasma on photometric systems.

Background

Liver: Total bile acids are metabolized in the liver and hence serve as a marker for normal liver function. Total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis, liver cancer, Cholestasis, Congenital and acquired vascular shunts.
Pregnancy: Obestetric cholestasis is the most common liver disease seen during pregnancy and the test key diagnostics tool for investigating suspected cases. the main symptom is itching, but tiredness , nausea and jaundice may also be reported. in addition to the discomfort experience by the mother, high levels of bile acids also increased risk to the baby and may lead to premature birth or greater risk of passing meconium in the womb.
Veterinary: In veterinary medicine the bile acids test is typically used to assess liver function in mammals such as cats, dogs, horses, pigs, cows and sheep. The test is also of use in the investigation of avian liver disease, where elevated liver enzymes do not always correlate with liver disease. In the most species a single, random sample is assessed but a dual sampling protocol is typically used with cats and dogs to investigate bile reabsorption. For the latter a fasting blood sample is taken and then a second, post prandial sample taken a few hours after being fed for comparison. Total bile acids are typically low in fasted state, whilst level will rise immediately after a meal. When the liver has normal mass and blood flow the bile acid levels will rapidly fall back to near fasting level as the liver removes them from the blood and returns them to the gall bladder.

Method

Enzymatic recycling method

Assay Principle

In the presence of Thio-NAD, the enzyme 3-á hydroxysteroid dehydrogenase (3-á HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-á HSD can convert 3-keto steroids and Thio-NADH to blie acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405nm.

Reagents

Reagent 1(R1)

Buffer (pH 7.0)	65mmol/L
Thio-NAD	1 g /L

Reagent 2(R2)

Buffer (pH 7.0)	65 mmol/L
Thio-NADH	6 g /L
Sodium Azide	<0.1%
3-a HSD	>5000 U/L

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability

Spectrum TBA reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles at 2 – 8 ^{o}C.

Deterioration

Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

The only acceptable anticoagulat is EDTA. Use preferably fresh serum.

Stability: 1 week at 4 – 8 ^{o}C
2 months at -20 ^{o}C

System Parameters

Wavelength	405 nm
Optical path	1 cm
Assay type	Fixed rate
Direction	Increase
Temperature	37 ^{o}C
Incubation time	5 minutes at 20–25^{o}C
Zero adjustment	Reagent Blank
Sensitivity	0.5 µmol/L
Linearity	150 µmol/L

Procedure

	Blank	Calibrator	specimen
Reagent1(R1)	300 µl	300 µl	300 µl
Calibrator	-------	5 µl	-------
Specimen	-------	-------	5 µl
Mix , incubate for 5 minutes at 37^{o}C then add:			
Reagent2(R2)	100 µl	100 µl	100 µl

Mix, and after 1 minute read the absorbance A1 of the Calibrator or specimen. After 3 minutes later, read absorbance A2 of calibrator or specimen.

Calculation

A2 – A1 = Aspecimen or Acalibrato or Ablank.

$$\text{TBA}(\mu\text{mol/L}) = \frac{A_{\text{specimen}} - A_{\text{blank}}}{A_{\text{Calibrator}} - A_{\text{blank}}} \times \text{conc. of calibrator}$$

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics
Precision

Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (µmol/L)	45.54	171.62
SD	0.35	0.65
CV%	0.77	0.38

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (µmol/L)	85.16	228.18
SD	3.25	6.69
CV%	3.82	2.93

Calibration

The assay requires the use of a total bile acids calibrator. Recalibration is recommended at anytime if one of the following events occurs:

*The Lot number of reagents changes.
* Preventative maintenance is performed or a critical component is replaced.
*Control values have shifted or are out of range and a new vial of control does not rectify the problem.

Methods Comparison

A comparison between Spectrum Diagnostics TBA reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.97 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay 0.5 µmol/L.

Linearity

The reaction is linear up to an albumin concentration of 180 µmol/L ; specimens showing higher concentration should be diluted 1+1 with physiological saline and repeat the assay (result × 2).

Interfering Substances

Haemolysis
A haemoglobin level of 150 mg/dL results in 13 % positive bias.

Icterus
No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia
No significant interference up to an intralipid level of 2 %.

Expected Values

0 -10.4 µmol/L
It is recommended that each laboratory should establish its own reference interval.

Analytical Range

0.5 – 180 µmol/L.

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Abbassi-Ghanavati M, Greer LG, cunningham FG.
2. Wallach, J.,Eighth ed. lippincott Williams & Wilkins
3. H. Bergmeyer, K. Gawehn, and M. Grassl in Methods of Enzymatic Analysis (Bergmeyer H. U. ed) 2nd Volume I, 505-507, Academic Press, Inc. New York, NY (1974).
4. Drummond, G. I. & Masanobu, Y. In: The enzymes (boyer, P.D. (ed.), (3rd ed.), vol.4, pp. 337(1971).

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
305 001	100 test
305 002	40 test

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