LiquiMAX Total Bile Acids

Enzymatic Cyclic Method

ORDER INFORMATION:

Ref. No.	Pack Size	Presentation
AVTBA-30	30 ml	Two reagents with calibrator

INTENTED USE:

LiquiMAX Total Bile Acids is an in-vitro diagonistic kit for the Quantitative Determination of Bile Acids in Human Serum. This kit is a automated.

INTENDED USER:

Laboratory technician

PRODUCT FEATURES:

Two liquid reagents with calibrator.

Serum is the Specimen.

Linear up to 150 µMol/L

3 Minutes Fixed Time Assay

Adaptable to Semi and Fully Auto Analyzers

CLINICAL SIGNIFICANCE :

Bile acids and their salts, usually sodium ones, are steroid acids found found predominantly in the bile of mammals. In humans, bile acid synthesis begins when liver cells synthesize the two primary bile acids, cholic and chenodeoxycholic acids by the cytochrome P450-mediated oxidation of cholesterol. The rate-limiting step is the addition of a hydroxyl group on position 7 of cholesterol by the enzyme cholesterol 7 alpha hydroxylase.

This enzyme is down-regulated by cholic acid and up-regulated by cholesterol. When these two bile acids are secreted into the lumen of the intestine, intestinal bacteria dehydroxylate a portion of each of them to form the secondary bile acids, deoxycholic acid (from cholic acid) and lithocholic acid (from chenodeoxycholic acid). All four of these bile acids can be taken back up into the blood stream, return to the liver, and be re-secreted in a process known as enterohepatic circulation.

All bile acids are found in human intestinal bile. The main function of bile acids is to facilitate the formation of micelles, which promotes processing of dietary fat; but also to eliminate cholesterol from body and driving the flow of bile to eliminate catabolites from the liver. Tests for bile acids are useful to diagnose a number of conditions, including cholestase, portosystemic shunt and hepatic microvascular dysplasia. Excess concentrations of bile acids in the colon are a cause of chronic diarrhea. It is well-documented that bile acids are carcinogens and tumor promoters in experimental models. Their role in carcinogenesis is best documented in Barrett's esophagus and adenocarcinoma at the gastroesophageal junctions.

PRINCIPLE

Bile Acids are converted by 3- -HSDH (3- -Hydroxysteroid dehydrogenase) into the corresponding ketons, in presence of thio-NAD. The thio-NAD reacts with NADH, giving thio-NADH yellow colour, with a max. absorbance at 405 nm.

The intensity of colour at the reaction conditions is directly proportional to the Bile Acids in the sample. Using the standard contained in the kit it is possible to prepare a Calibration Curve to refer. Plotting on the Calibration Curve absorbance values and concentration for each single sample, may be determined the concentration of each sample.

STORAGE & STABILITY:

All the Reagents are stable up to the expiry date mentioned on the labels when properly stored at stored at 2-8°C.

KIT COMPONENTS

1. TBA Reagent R1
2. TBA Reagent R2
3. TBA Calibrator : Concentration as stated on the label

COMPOSITION

All reagents are ready to use

R1 - BUFFER	Phosphate buffer	> 10 mmol/L
	Thio-NAD	> 0.1 mmol/L
R2 - 3-Alpha-HSDH	3-Alpha-HSDH	> 50 U/L
	NADH	> 0.1 mmol/L
R3 - CAL	Solution of Bile Acids = 100 µMol/L NaN3 < 0.1%	

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN COLLECTION AND STORAGE

Fresh Serum without Hemolysis

Samples collection incompliance with CLSI (NCCLS)

The sample can be stored at 2-8°C, up to 6 days.

SYSTEM PARAMETERS

Reaction Type	:	Fixed Time
Wave Lenth	:	405nm
Delay Time	:	60 Sec.
Measuring Time	:	120 Sec.
Reagent Volume	:	R1-400 µL + R2-100 µL
Reagent Volume	:	R1-400 µL
R2-100 µL	:	10 µL
Flo Cell Temp.	:	37° C
Low Normal	:	0
High Normal	:	12
Unit	:	µmol/L
Linearity	:	200

TEST PROCEDURE

Pipette in test tubes labeled as :

Reagent	Calibrator	Sample
R1	360 µL	360 µL
Calibrator	5 µL	----
Serum Sample	----	5 µL
R2	120 µL	120 µL

Read absorbances of Calibrator and Sample against Distilled Water Blank at 405 nm as follows:

Initial absorbance A_0	=	Exactly after 60 Sec.
Final absorbance A_1	=	Exactly 120 Sec. After A_0

CALCULATIONS:

Determine △Abs for S and T

$$\triangle \text{ Abs for S} = \text{Abs } S_1 - \text{Abs } S_0$$

$$\triangle \text{ Abs for T} = \text{Abs } T_1 - \text{Abs } T_0$$

$$\text{Bile Acids Conc. } \mu\text{Mol/L} = \frac{\text{Abs. of Sample}}{\text{Abs. of Calibrator}} \times \text{Calibrator Concentration}$$

EXPECTED VALUES:
Normal Values Bile Acids: 0-12 µMol/L
Since the normal values depend on age, sex, diet, geographic area and other factors, each laboratory should establish its own normal values for this procedure.

QUALITY CONTROL & CALIBRATION
It is recommend to perform internal quality control with assayed normal (Randox) and assayed abnormal (Randox), to confirm the validity of the test and assure the accuracy of patient result. Using the recommended calibrator (Avecon) or the standard included, calibrate the assay:
a) When using a new reagent or lot.
b) When QC values are out of range.

PERFORMANCE CHARACTERISTICS
1. Linearity
Linearity : 200 µMol/L

2. Sensitivity/ Limit of Detection (LOD)
The lower limit of detection is 0.1 µMol/L

3. Interferences
Interference test criterion: recovery ± 10% of initial value.
No interference found on samples with: - total bilirubin up to 40 mg/dL; haemoglobin up to 600 mg/dL; lipemia [Intralipid ®] up to 4000 mg/dL; ascorbic acid up to 50 mg/dL.

4. Precision:

Intra -Assay	Mean	SD	CV
n = 40	µMol/L	µMol/L	**[%]**
Sample 1	13.4	0.24	1.81
Sample 2	58.9	0.60	1.01
Sample 3	103	1.50	1.45

Inter -Assay	Mean	SD	CV
n = 40	µMol/L	µMol/L	**[%]**
Sample 1	13.4	0.24	1.81
Sample 2	58.9	0.49	0.80
Sample 3	103	0.65	0.63

5. Method Comparison:
Linear regression equation y = 1.0140x - 1
Correlation coefficient r = 0.9997 n = 20

LIMITATIONS
Measuring range: 0.1-200 µMol/L. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL
Reagents must be disposed off in accordance with local regulations.

NOTES
1. This product has been formulated for in vitro diagnostic use.
2. A proportional variation of the reaction volumes does not change the result.
3. DO NOT mix Reagents from different Production lots.
4. For concentration of bile acids higher than 200 mol/L, dilute the sample 1:4 with saline solution, repeat the determination and multiply the result by 4.
5. In addition to the possible risk indications, the Reagent cancontain preservatives (as sodium azide or others), which total concentration is lower than the limits mentioned in Dir. 67/548/CEE e 88/379/CEE and following modifications regarding classification, labelling and packaging of dangerous preparations (Reagents). However it is recommended to handle the reagents carefully, avoiding ingestion and contact with eyes, mucous membranes and skin; to use reagents according to good laboratory practice. On the material safety data sheet are detailed the operating procedures for the manipulation of this product. Material safety data sheet should be supplied on request.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000).
3. Mashige F. et al., Clin. Chem. 27/8, 1352 (1981)

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

Manufactured in India by :
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