BILE ACIDS

REF 450-A

For other languages	Για τις άλλες λώσσες
Pour d'autres langues	For andre sprog
Für andere Sprachen	Pro jiné jazyky
Para otras lenguas	For andre språk
Per le altre lingue	Diğer diller için
Para outras línguas	

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INTENDED USE

Trinity Biotech Bile Acids reagents are intended for the quantitative, enzymatic determination of bile acids concentration of serum at 530 nm.

SUMMARY

Bile acids are present in most body fluids with the highest concentrations being found in bile[1]. The major bile acids are cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid[2]. Bile acids, after conjugation with glycine or taurine and sometimes with glucuronide or sulfate esters, are secreted into bile and undergo an enterohepatic circulation. Serum bile acids originate from the intestinal absorption of bile acids.

An increase in serum bile acids concentration in the fasting state or post-prandial is considered to be a specific indicator of liver disease[3]. A decreased level indicates bile acid malabsorption, possibly due to ileal dysfunction[4]

Bile acids concentration can be measured by chromatographic[5], spectrophotometric[6-8], or fluorometric[9] procedures. They can also be determined by radioimmunoassay[10] or similar competitive binding techniques, as well as by bioluminescence detection methods.[2] Trinity Biotech Bile Acids reagents measure serum bile acids concentration enzymatically. The methodology is based essentially on the procedure of Mashige et al.[7]

PRINCIPLE

The enzymatic reactions involved in the bile acids determination are as follows:

3α-Hydroxybile Acids + NAD $\xrightarrow{\text{3 α-HSD}}$ 3-Oxo Bile Acids + NADH

NADH + NBT $\xrightarrow{\text{Diaphorase}}$ NAD + Formazan

Bile acids are first oxidized to 3-oxo bile acids in the reaction catalyzed by 3α hydroxysteroid dehydrogenase (3αHSD). During this oxidative reaction, an equimolar quantity of nicotinamide adenine dinucleotide (NAD) is reduced to NADH. The NADH is subsequently oxidized to NAD with concomitant reduction of nitro blue tetrazolium salt (NBT) to formazan by the catalytic action of diaphorase. The formazan has an absorbance maximum at 530 nm. The intensity of the color produced is directly proportional to bile acids concentration in the sample.

REAGENTS

The Bile Acids reagents, when reconstituted according to the directions, contain approximately the following concentrations of active ingredients:

BILE ACIDS REAGENT A, Catalogue No. 450-1,16 x 10 ml

NAD	2.5 mmol/L
NBT	0.61 mmol/L
Diaphorase	625 U/L
Buffer	pH 7.0 ± 0.1

Nonreactive stabilizers and fillers.

BILE ACIDS REAGENT B, Catalogue No. 450-2, 4 x 5 ml

3αHSD	1250 U/L

Nonreactive stabilizers and fillers.

PRECAUTIONS:

The reagents are for "*in vitro* diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.
For professional use only.
In case of damage, do not use.

Avoid contact and inhalation of Bile Acids Reagents A and B.

Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

The following instruction should be adhered to when opening the red flip-seal cap as it has a sharp edge after opening:

- A tweezers, needle-nose pliers, forceps, de-cappers, spatula or similar type of object should be used to open and peel off the flip-seal from the vial. When doing this action, ensure it is done outwards, away from the body.
- Latex gloves should also be worn to provide further protection to the user.

PREPARATION:

Reconstitute Bile Acids Reagent A and Bile Acids Reagent B with volume of deionized water indicated on the labels. After addition of water, stopper vials and immediately mix several times by inversion. DO NOT SHAKE.
Prepare Test Reagent and Blank Reagent from the same pools of Bile Acids Reagent A. Combine 4 ml of Bile Acids Reagent A with 1 ml Bile Acids Reagent B to prepare Test Reagent. Prepare Blank Reagent by combining 4 ml Bile Acids Reagent A with 1 ml deionized water. If reagents are to be used in an automated instrument, please refer to respective application procedures for reagent preparation instructions.

STORAGE AND STABILITY:

Store Bile Acids Reagent A and Bile Acids Reagent B refrigerated (2-8°C). The reagents are stable until the expiration date shown on the respective labels.

Store Bile Acid Calibrators refrigerated (2-8°C). The reagents are stable until the expiration date shown on the respective labels.

Store Bile Acid Stop Reagent at room temperature (18-26°C). The reagents are stable until the expiration date shown on the respective labels.

Reconstituted Bile Acids Reagent A is stable for 24 hours at room temperature (18-26°C) and for 1 week refrigerated (2-8°C). Bile Acids Reagent B is stable for 1 day at room temperature (18-26°C) and for 1 week refrigerated (2-8°C).

Test Reagent and Blank Reagent are stable for 24 hours at room temperature (18- 26°C) and for 3 days refrigerated (2-8°C).

DETERIORIATION:

The reconstituted reagents are not suitable for use if the reagents show an initial absorbance greater than 0.3 when measured in a 1-cm lightpath at 530 nm vs water as a reference.

Discard the vials if dry Bile Acids Reagent A or Bile Acids Reagent B exhibit caking due to possible moisture penetration, do not dissolve completely or if solutions appear turbid.

DISCRETE ANALYSER APPLICATIONS

Please contact Trinity Biotech Technical Services Department for more information regarding applications procedures for Bile Acids.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

After patients have fasted overnight, collect blood in tubes containing no anticoagulants. Centrifuge samples promptly after clot formation to obtain clear serum. Store samples refrigerated (2-8°C) for 7 days or freeze at -20°C for 4 months if analysis is not performed the same day.

INTERFERING SUBSTANCES: Extremely lipemic, haemolysed and icteric serum may give misleading results. In such instances, it is recommended that the sample be diluted prior to use.

MANUAL PROCEDURE

MATERIALS PROVIDED:
Bile Acids Reagent A
Bile Acids Reagent B

MATERIALS REQUIRED BUT NOT PROVIDED:

- Spectrophotometer, with temperature controlled cuvette compartment, capable of accurately measuring absorbance at 530 nm
- Cuvettes with optical properties suitable for use at 530nm
- Pipetting devices for the accurate delivery of volumes required for the assay
- Bile Acids Calibrator, Catalogue No. 450-100 or 450-11
- Bile Acids Stop Reagent, Catalogue No. 450-3

PROCEDURE:

1. Prepare Bile Acids reagents according to instructions.
2. Set spectrophotometer wavelength at 530nm and absorbance reading to zero with water as reference.
3. Warm Test Reagent and Blank Reagent to 37°C.
4. Set up a series of labeled tubes for Calibrator, Calibrator Blank, Control, Control Blank, Sample and Sample Blank.
5. Pipette 0.2 ml each of Calibrator, Control or Sample to corresponding test tubes.
6. At timed intervals, add 0.5 ml Test Reagent and 0.5 ml Blank Reagent to corresponding tubes.
7. Mix immediately and incubate at 37°C for five minutes.
8. Read absorbance of all tubes at 530nm using the same timed intervals from Step 6.

   NOTE: Alternatively, stop enzyme reaction by adding 0.1 ml Bile Acids Stop Reagent, Catalogue No. 450-3, using the same timed intervals from Step 6. The color, after stopping the reaction, is stable for at least 1 hour.
9. Subtract Blank absorbance from corresponding Test absorbance to obtain absorbance change due to bile acids in Calibrator, Control and Sample.
10. To determine bile acids concentration (µmol/L) in sample or control, refer to "Calculations" section.

CALIBRATION:

Bile acids concentration in samples can be calculated using a Bile Acids Calibrator, Catalogue No. 450-100. The bile acids concentration in samples can also be derived from a calibration curve prepared using multi-level Bile Acids Calibrators, Catalogue No. 450-11. Trinity Biotech Bile Acids procedure is linear to 200µmol/L.

BILE ACIDS CALIBRATOR SET, Catalogue No. 450-11 & 450-100

EACH 450-11 SET CONTAINS
5 µmol/L – 5ml
25 µmol/L – 5ml
50 µmol/L – 5ml
100 µmol/L – 5ml
200 µmol/L – 5ml

EACH 450-100 SET CONTAINS
100 µmol/L – 5ml

Solutions contain bile acids in bovine serum base with sodium azide, 0.1%, as preservative.

PRECAUTIONS:

Bile Acids Calibrators are for "*in vitro* diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.
For professional use only.
In case of damage, do not use.

PREPARATION:

Bile Acids Calibrators are supplied ready for use.

STORAGE AND STABILITY:

Store Bile Acids Calibrators refrigerated (2-8°C). Vial labels bear expiration date.

QUALITY CONTROL:

The reliability of test results should be monitored by routine use of control sera with known bile acids concentration such as Trinity Biotech Bile Acids Control Set, Catalogue No. 450-22. Bile Acids concentration determined by this procedure should fall within the range stated for the controls.

Quality Controls should fall within their assigned ranges; otherwise the test run is invalid.

CALCULATIONS

Determine bile acids concentration in sample as follows:

$$\text{Serum Bile Concentration (µmol/L)} = \frac{AST-ASB}{ACT-ACB} \text{ x Concentration of Calibrator}$$

Where:

AST = Absorbance of Sample with Test Reagent
ASB = Absorbance of Sample with Blank Reagent
ACT = Absorbance of Calibrator with Test Reagent
ACB = Absorbance of Calibrator with Blank Reagent

EXAMPLE:

Where a serum sample was assayed for bile acids concentration by the above procedure, the following absorbance values were obtained:

AST = 0.317
ASB = 0.075
ACT = 0.475
ACB = 0.072

$$\text{Serum Bile Acid Concentration (µmol/L)} = \frac{0.317 - 0.075}{0.475 - 0.072} \times 100^* = 60.0$$

$$\frac{0.317-0.075}{0.475-0.072} \times 100^* = 60.0$$

* Bile Acids Concentration (µmol/L) of Calibrator

LIMITATIONS

Trinity Biotech Bile Acids reagent is linear to 200 µmol/L. If concentration exceeds upper limit of linearity, dilute 1 part sample with 1 part isotonic saline and reassay. Multiply result by 2 to compensate for dilution.

EXPECTED VALUES

The expected range of serum bile acids concentration is dependent on the measurement method, a previous study using this test chemistry on samples obtained from 45 apparently healthy adult males and females fasted overnight determined an expected range of 0-8.1 µmol/L. A further study on the serum of 49 fasting adults (20 men and 29 women) ranged from 1-7 µmol/L (mean 3.5 µmol/L, 2 SD 3.5 µmol/L)[7].
It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

PERFORMANCE CHARACTERISTICS

COMPARISON:

A total of 50 samples were analysed on three separate clinical chemistry platforms, the following details the observed correlation

Platform Analyser 1

"A total of 50 serum specimens with bile acids concentrations ranging from 7.7 – 107.2 µmol/L was assayed by the described method and by a similar procedure. Comparison of bile acids concentrations obtained by both the procedures yielded a correlation coefficient of 0.995 and the regression equation was y =1.1639x + 3.1417."

Platform Analyser 2

"A total of 50 serum specimens with bile acids concentrations ranging from 6.5 - 102.8 µmol/L was assayed by the described method and by a similar procedure. Comparison of bile acids concentrations obtained by both the procedures yielded a correlation coefficient of 0.996 and the regression equation was y = 1.2164x - 2.151."

Platform Analyser 3

"A total of 50 serum specimens with bile acids concentrations ranging from 8.9 - 105.8 µmol/L was assayed by the described method and by a similar procedure. Comparisons of bile acids concentrations obtained by both the procedures yielded a correlation coefficient of 0.997 and the regression equation was y = 1.2156x + 2.8104."

SENSITIVITY:

An absorbance change of 0.003 corresponds to a bile acids concentration of 1 µmol/L when a spectrophotometer typically found in clinical laboratories is used for the measurement under the stated assay conditions.

PRECISION:

For Intra Precision, twenty replicate assays were run on three serum samples of varying bile acids concentration on each of three platform analysers. For Inter Precision, three serum samples of varying bile acids concentration were run on twenty separate occasions in duplicate on each of three platform analysers.

	Platform Analyser 1			Platform Analyser 2			Platform Analyser 3		
	Intra Precision								
Sample	1	2	3	1	2	3	1	2	3
Mean Bile Acids (µmol /L)	7.9	36.0	134.2	9.4	37.6	142.6	9.5	38.6	133.4
Standard Deviation	0.4	0.4	0.4	0.2	0.4	1.4	0.2	0.2	0.7
%CV	4.7	1.0	0.3	2.0	1.0	1.0	2.0	0.4	0.5
No. Of Assays	20	20	20	20	20	20	20	20	20
	Inter Precision								
Sample	1	2	3	1	2	3	1	2	3
Mean Bile Acids (µmol /L)	6.5	35.4	114.3	6.7	36.7	121.4	4.7	32.3	112.4
Standard Deviation	1.0	2.9	4.8	1.1	2.8	6.3	0.3	1.9	5.5
%CV	15.2	8.1	4.2	16.0	7.5	5.3	7.0	6.0	4.9
No. Of Assays	40	40	40	40	40	40	40	40	40

Trinity Biotech warrants that its products conform to the information contained in this and other Trinity Biotech publications. Purchaser must determine the suitability of this product for its particular use.

REFERENCES

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3. Gilmore IT, Hofmann AF: Altered drug metabolism and elevated serum bile acids in liver disease: A unified pharmacokinetic explanation. Gastroenterology 78:177, 1980
4. Heubi JE, BalistreriWF, Partin GP, SchubertWK, Suchy FJ: Enterohepatic circulation of bile acids in infants and children with ileal resection. J Lab Clin Med 95:231, 1980
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10. Miller P, Weiss S, Cornell M, Dockery J: Specific 125 I radioimmunosassay of conjugated cholylglycine, a bile acid in serum. Clin Chem 27:1698, 1981

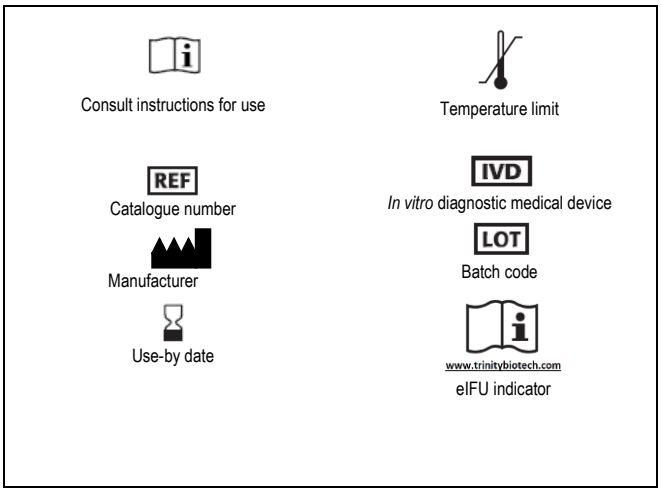
ORDERING INFORMATION

KIT		450-A
Kit Content	**Item**	**Quantity**
450-1	Bile Acids Reagent A,	16x10ml
450-2	Bile Acids Reagent B,	4x5 ml

ADDITIONAL PRODUCTS AVAILABLE

Catalogue No.	Item	Quantity
450-100	Bile Acid Calibrator – 100 µMOL/L	5 ml
450-3	Bile Acid Stop Reagent	50 ml
450-150	Bile Acid Reagent A	50ml
450-225	Bile Acid Reagent B	25ml
450-2	Bile Acid Reagent B	5ml
450-11	BILE ACIDS CALIBRATOR SET Set contains 5 ml each of the following calibrators with bile acids concentrations of 5, 25, 50, 100 and 200 µmol/L	5x5 ml
450-22	BILE ACIDS CONTROL SET Set contains 3x5 ml each of a normal and abnormal level.	6x5 ml

GUIDE TO SYMBOLS



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