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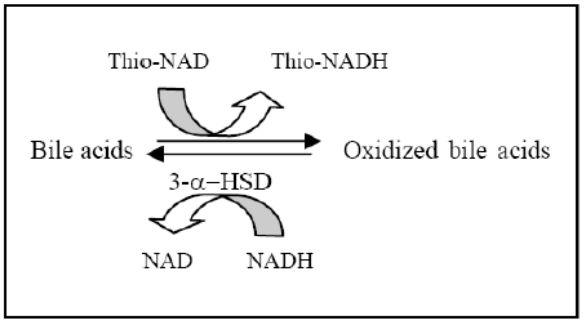
Total Bile Acids Assay Kit (Enzymatic Cycling)
Catalog Number: BQ 042A-EALD

Intended Use
The BQ Kits Total Bile Acids Assay Kit is intended for the *in vitro* quantitative determination of serum total bile acids (TBA) in human serum samples. Total bile acids are metabolized in the liver and serve as a marker for normal and abnormal liver function. Serum total bile acids are increased in patients with liver disease.

Clinical Significance [1,2]
Total bile acids are metabolized in the liver and hence, serve as a marker for normal liver function. Serum total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer.

Assay Principle
The reagents of the assay kit are in a stable liquid formulation that allows ease of use coupled with enhanced performance characteristics. 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 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.



Specimen Collection and Handling
Use fresh patient serum samples. TBA concentration is increased after meals; hence, sample should be collected under fasting condition. EDTA treated or Lithium heparin plasma samples are suitable for use. Serum or plasma samples are stable for a 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 the TBA Assay.

Reagent Composition

Reagent	Composition	Volume
R1	Thio-NAD >0.1 mM, Buffer	2 x 60 mL
R2	3-α-HSD >2kU/L, NADH >0.1 mM, Buffer	2 x 20 mL
Calibrator	Conjugated cholic acids, Buffer	1 x 2 mL

Reagent Preparation
Reagents are ready-to-use, liquid reagents. The intrinsic yellow to yellow-brown color of the TBA reagent does not interfere with the test.

Reagent Stability and Storage
Unopened reagents are stable until the expiration date printed on the label. Reagents are light sensitive and should be stored at 2-8 °C. Reagents from different lots must not be interchanged.

Materials Required But Not Provided
An analyzer capable of dispensing two reagents and of measuring absorbance at 405 nm with temperature control (37°C).

Precautions
1. For *in vitro* diagnostic use.
2. Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid swallowing and contact with skin or mucous membranes.

Assay Procedure
Application sheets for use of the Total Bile Acid Enzymatic Assay on automated clinical chemistry analyzers are attached.

Manual Procedure

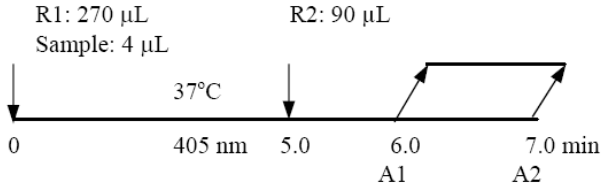
1. Pipette 270µl R1 into cuvette, to which 4µl of sample, standard, or water (as blank) is added.
2. Incubate at 37°C for 3 minutes and blank (autozero) absorbance at 405 nm.
3. Pipette 90µl of R2 into the cuvette, mix and immediately monitor the absorbance at 405nm for 2 minutes.
4. Calculate ΔA405/min for sample, blank, and standard by subtracting O.D. value at 60 seconds from O.D. value at 120 seconds.
   ΔA405/min = (O.D. at 120sec – O.D. at 60 sec).
5. Determine total bile acids concentration using the equation below:

Sample (TBA, µmole/L) =

$$\frac{\text{Sample } \Delta A405\text{nm/min} - \text{Blank } \Delta A405\text{nm/min}}{\text{Standard } \Delta A405\text{nm/min} - \text{Blank } \Delta A405\text{nm/min}} \times \text{Standard}$$

If samples bile acids exceed linear range (1-180 µmole/L), dilute samples with 0.9% NaCl before assay.

Assay Scheme for Chemistry Analyzers



Calibration
A bile acids calibrator is included with the reagents and, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure. Calibration frequency may vary and is dependent on instrument application.

Results
Bile acids concentration is expressed as µmole/L (µEq/L).

Reference Range [3]
Serum or plasma containing 0-10 µmole/L bile acids is considered normal range. We suggest that each laboratory establish a range of normal values for the population in their region.

Quality Control
We recommend that each laboratory use bile acid controls to validate the performance of the bile acid reagents. A set of normal and abnormal ranges bile acid controls is available from BQ Kits. If the results from the controls fall outside the acceptable limits, as determined by the manufacturer, the tests should not be performed. We recommend that your quality control testing follows federal, state and local guidelines or accreditation requirements.

Limitations
- A sample with a bile acids level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.
- Specimens from patients, who are on Ursodeoxycholic Acid (UDCA) treatment, are not suitable for use with the TBA Assay.

Performance Characteristics (Hitachi 717)
These performance characteristics were determined using automated procedures on Hitachi 717.

Accuracy
The performance of this assay was compared with the performance of a similar total bile acids assay on the Hitachi 717 analyzer using serum samples.

Fifty two (52) serum samples ranging from 0.47 – 131.25 µmole/L gave a correlation coefficient of 0.9918. Linear regression analysis gave the following equation:

This method = 1.1536 (reference method) – 0.8567 µmol/L

A matched set of serum and lithium heparin plasma samples ranging from 0.14 – 21.18 µmol/L gave a correlation coefficient of 0.9805. Linear regression analysis gave the following equation:

Lithium heparin = 0.9972 (serum) + 0.1178 µmol/L

Precision Studies
The intra-assay precision and inter-assay precision were evaluated in samples containing two different bile acid levels (8 µM and 23 µM). The inter-assay precision was evaluated by testing these two level specimens (low = 8 µM and high = 23 µM) in 20 runs. All tests were done using the Hitachi 717 Auto-analyzer instruments.

Precision data is summarized in the table below:

Intra-Assay Precision

	Level 1 (8 µM)	Level 2 (23 µM)
Number of Replicates	20	20
Mean	7.93	23.5
SD	0.31	0.3
CV%	3.9%	1.3%

Inter-Assay Precision

	Level 1 (8 µM)	Level 2 (23 µM)
Number of Replicates	20	20
Mean	8.12	23.0
SD	0.24	0.61
CV%	2.9%	2.6%

Linearity
Linearity studies using a Hitachi 717 analyzer showed that the Total Bile Acids assay has a linear range from 0 to 180 µM.

Interference
Interference for the total bile acids assay was evaluated on a Hitachi 717 analyzer. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides at 750 mg/mL, Ascorbic acid at 50 mg/dL, Bilirubin at 50mg/dL and Homoglobin at 500mg/dL.

References

1. LaRusso, 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 Guide to Laboratory Tests. 4th ed. St. Louis, MO: Saunders/Elsevier, 2006. 170-171.

HITACHI 911 TBA cycling Parameters

HITACHI 911 Parameters	TBA (cycling)
Test	(TBA)
Assay Code	(2 Point Rate) (10) ( )
Assay Point (Serum) (Urine)	(20) - (26) - (0) - (0)
Wavelength (2nd/Primary)	(600) / (415)
S. Vol (Normal)	(4) (0) (0) (10) (0) (0)
S. Vol (Decrease)	(4) (0) (0) (10) (0) (0)
S. Vol (Increase)	(4) (0) (0) (10) (0) (0)
ABS. Limit	(3200) (0) (Increase)
Prozone Limit	(-3200) (3200) (Lower)
Reagent T1	(270) (0) (00301) (0)
T2	(0) (0) (00301) (0)
T3	(90) (0) (00301) (0)
T4	(0) (0) (00301) (0)
Calibration Type	(Linear) (2) (2) (0) ( )
Auto Time Out Blank	0
Span	0
2 Point	0
Full	0
SD Limit	0.1
Duplicate Limit	3200
Sensitivity Limit	100
S1 ABS Limit	(-3200) (3200)

HITACHI 717 Parameters	TBA(cycling)
Test	TBA
Assay Code	Rate A
Assay Point	(30)-(35)
Wavelength	600/405
Calibration Method	Linear
Unit	µmol/L
Sample volume	(4)(4)
Reagent vol. R1	(270)(100)(NO)
Reagent vol. R2	(90)(100)(NO)
STD (1) CONC. –POS	(0)-(1)*
STD (2) CONC. –POS	(50)-(2)*
ABS.Limit	32000-Increse
Expected value (normal Value)	0-10
Tech Limit	0-180
Standard position	*
Standard Conc.	50.0
Water position	*
Water Conc.	0.0

Attention: * Entered By Operator
**Each reading cycle is 12 seconds.

HITACHI 917 Parameters	TBA(cycling)
Test	TBA
Assay/Point	Rate A
	[10][21][25][0][0]
Wavelength(Sub/Main)	[600][405]
Sample volume (Normal)	[4.0][0][0]
Sample volume (Dec.)	[10][0][0]
Sample volume (Inc.)	[10][0][0]
Diluent	[water][0]
Reagent vol. R1	[270][0][10003][0]
Reagent vol. R2	[0][0][10003][0]
Reagent vol. R1	[90][10003][0][0]
Reagent vol. R2	[0][0][10003][0]
ABS.Limit	[32000][Increase]
STD (1) Conc.	[0]
POS.	[*]
STD (2) Conc.	[50]
POS	[*]

Attention: * Entered By Operator
Hitachi 917: **Each reading cycle is 18 seconds.

Cobas Mira S Parameters	TBA cycling
Measurement Mode	Absorb
Reaction Mode	R-S-SR1
Calibration Mode	Slope Avg
Reagent Blank	Reag/DIL
Cleaner	No
Wavelength	405 nm
Decimal position	3
Unit	µmol/L
Sample cycle	1
Sample volume	4.0 uL
Sample dilution	H_2O
Dilution volume	0.0 uL
Reagent cycle	1
Reagent volume	270 µL
Dilution volume	0.0 µL
Start R1 cycle	10
Reagent volume	90 µl
Dilution volume	0.0 µL
Sample limit	No
Reaction direction	Increase
Convers. Factor	1.0000
Offset	0.0000
Test range low	0.000 µmol/L
Test range High	160.00 µmol/L
Number of steps	1
Calc. Step A	Kinetics
Readings first	13
Readings last	16
Calibration	
Cali. Interval	Each day
Time	No
Blank	
Reagent range low	0.0
high	0.4
Blank range low	0.0
high	0.1
Standard pos	1
Standard-1	50.0µmol/L
Replicate	Dupl

Note: Each cycle is 25seconds on the COBAS MIRA S analyzer.

** These reagent parameters have **not been fully validated** for this analyzer. The parameters are based on BQ Kits knowledge of the analyzer and BQ Kits reagents should perform adequately. **However, you should use these parameters as guidelines in conjunction with your Quality control program for validation. ****

Olympus AU400/640
Temperature = 37°C

Use the following parameters with calibrator for calibration.

General

Test Name: TBA Type: Serum Operation: Yes

Sample Volume 4.0 µL Dilution 0 µL Pre-Dilution Rate 1

Reagents: Min OD Max OD

R1 volume 270 µL Dilution 0 µL L: -2.000 H: 2.500
R2 volume 90 µL Dilution 0 µL

Wavelength: Pri. 410 Sec. 700 Reagent OD Limit:

Method: Rate First L:-2.000 First H: 2.500
Reaction Slope: + Last L: -2.000 Last H: 2.500

Measuring Point 1: First 14 Last 18 Dynamic Range:
Measuring Point 2: First Last L: 0.00 H: 999.0

Linearity 20% Correlation Factor:
No-Lag-Time: No A: 1.0000 B: 0.0000

Onboard stability Period:

Calibration Type AB Formula: Y=AX+B
Counts 2 Process: CONC

	Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point	1	1	50.00	-999999.0	9999999
Point	2				

Advanced Calibration: No
MB Type Factor: Calibration Stability Period: 999

Olympus AU 680 Parameters
Temperature = 37°C

Use the following parameters with calibrator for calibration.

General
Test Name: TBA Type: Serum Operation: Yes

Sample: Volume 3.0 µL Dilution 0 µL Pre-Dilution Rate: 1
Min OD -2.0 Max OD: 2.5
Reagents:
R1 volume: 180 µL Dilution: 0 µL L: -2.000 H: 2.500
R2 volume: 60 µL Dilution: 0 µL

Wavelength: Pri. 410 Sec. 700 Reagent OD Limit:
Method: RATE First L:-2.000 First H: 2.500
Reaction Slope: + Last L: -2.000 Last H: 2.500

Measuring Point 1: First 14 Last 18 Dynamic Range:
Measuring Point 2: First Last L: -99999 H: 99999

Linearity: 20% Correlation Factor:
No-Lag-Time: A: 1.000 B: 0.0000
Onboard stability Period: 999

Calibration Type: AB Formula: Y=AX+B Counts 2 Process: CONC

	Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1	1		*	-2.0	2.5
Point 2	2		*	-2.0	2.5

Advanced Calibration: No
MB Type Factor: Calibration Stability Period: 999

* = Value input by Operator

** These reagent parameters have **not been fully validated** for this analyzer. The parameters are based on BQ Kits knowledge of the analyzer and BQ Kits reagents should perform adequately. **However, you should use these parameters as guidelines in conjunction with your Quality control program for validation before use. ****