Total bile acids 21 FS*

Order Information

Cat. No.	Kit size
1 2238 99 10 930	R1 4 x 12 mL + R2 2 x 8 mL

Intended Use

Diagnostic reagent for quantitative in vitro determination of total bile acids in human serum on automated photometric systems.

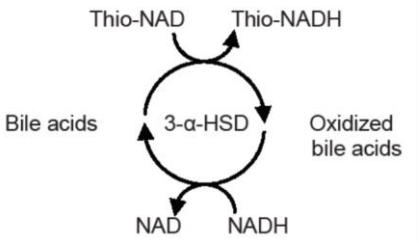
Summary

Bile acids (BA) are water soluble, amphipathic end products of the cholesterol catabolism which are synthesized in the liver, stored in the gall bladder and secreted in the intestine during digestion [1,2]. Throughout this metabolism, BA change their form from primary via secondary to tertiary BA and their conjugates. Total bile acids (TBA) refer to the sum of all these forms. Serum TBA levels are a sensitive marker of liver function, reflecting hepatic synthesis, secretion and re-absorption [2,3]. Compared to conventional liver screening tests such as ALT or AST, which indicate acute liver damage, the determination of total bile acids allows early detection of liver dysfunction and early treatment and prevention of severe, irreversible liver damage. Once a patient suffers from a liver disease, serum TBA can be used to monitor the treatment response [4-6]. Although TBA levels provide early diagnosis of hepatobiliary deficiencies, they do not allow the differentiation between various diseases. Increased serum TBA levels are associated with several diseases such as acute and chronic hepatitis, intrahepatic cholestasis of pregnancy (ICP), liver sclerosis, cirrhosis, and cancer [2-9]. The determination of TBA levels in pregnant women is considered to be the most important biomarker for diagnosis and monitoring of ICP, also known as obstetric cholestasis [10-12]. ICP is the most common liver disease that occurs during pregnancy; usually during the last 3 months of pregnancy. It is caused by a reversible, hormonally bile secretion disturbance which leads to a restricted bile flow through the gallbladder and in turn, to an accumulation of bile acids in the liver and possibly in the bloodstream [7,13]. Pregnancy-cholestasis is characterized by strong itching (pruritus) [11]. During ICP, TBA levels may rise up to 220 µmol/L [12], leading to an increased risk of fetal distress, premature birth or even stillbirth. TBA concentrations above 40 µmol/L may be fetotoxic [11]. Decreased serum TBA levels are associated with ileal dysfunction, malabsorption, diarrhea or Crohn's disease. In the veterinary field, serum TBA measurements are also of common practice [14].

Method

Enzymatic cycling method

Two reactions are combined in the new generation enzymatic cycling method. In the presence of Thio-NAD, the enzyme 3-α-hydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-ketosteroids and Thio-NADH. The reaction is reversible and 3-α-HSD can convert 3-ketosteroids and NADH to bile acids and NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring the specific change of absorbance at 405 nm. This cycling reaction leads to significant signal amplification. [15]



Reagents

Components and Concentrations

R1:	Buffer	
	Thio-NAD	> 0.1 mmol/L
R2:	Buffer	
	3-α-HSD	> 2 kU/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

Reagents are temperature-sensitive. Avoid discontinuity of the cold chain.

The in-use stability of the reagent is 15 months.

Warnings and Precautions

1. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 contains material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. Postprandial serum TBA levels are generally higher than fasting serum TBA levels. Thus, fasting samples should be used for bile acid determination [3].
4. In very rare cases, samples of patients with gammopathy might give falsified results [16].
5. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
6. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
7. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
8. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum (fasting > 12h)

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Samples from patients under bile acid analogues treatment such as fusidic acid, ursodeoxycholic acid or obeticholic acid are unsuitable for analysis [17].

Stability:

1 day	at	20 – 25°C
1 week	at	2 – 8°C
1 year	at	–20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	410/596 nm
Temperature	37°C
Measurement	Kinetic
Sample/Calibrator	1.3 µL
Reagent 1	90 µL
Reagent 2	30 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance	Cycle 25/32 (367 s/464 s)
Calibration	Linear

Calculation

With Calibrator

$$\text{Bile acids [µmol/L]} = \frac{\Delta A/\text{min. Sample}}{\Delta A/\text{min. Cal.}} \times \text{Conc. Cal. [µmol/L]}$$

Calibrators and Controls

DiaSys TruCal TBA is recommended for calibration. Calibrator values have been made traceable to a commercially available measurement procedure. Use DiaSys TruLab N and P for internal quality control. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal TBA	1 2240 99 10 037	3 x 1 mL
TruLab N	5 9000 99 10 061	6 x 5 mL
	5 9000 99 10 062	20 x 5 mL
TruLab P	5 9050 99 10 061	6 x 5 mL
	5 9050 99 10 062	20 x 5 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range up to 220 µmol/L.
When values exceed this range samples should be diluted 1 + 5 with NaCl solution (9 g/L) and the result multiplied by 6.

Limit of detection**	2 µmol/L

Interfering substance	Interferences ≤ 10% up to	Analyte concentration [µmol/L]
Ascorbic acid	100 mg/dL	8.56
	100 mg/dL	23.3
Bilirubin (conjugated)	60 mg/dL	8.18
	60 mg/dL	24.4
Bilirubin (unconjugated)	60 mg/dL	8.73
	60 mg/dL	25.0
Hemoglobin	400 mg/dL	7.78
	800 mg/dL	25.1
Lipemia (triglycerides)	700 mg/dL	8.25
	2000 mg/dL	26.4
Sulfapyridine	350 mg/L	8.42
	350 mg/L	25.5
Sulfasalazine	350 mg/L	7.08
	350 mg/L	24.4
Temozolomide	30 mg/L	7.82
	30 mg/L	25.3

For further information on interfering substances refer to Young DS [18,19].

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/L]	5.41	10.2	199
CV [%]	2.38	0.829	0.598
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/L]	5.39	10.4	201
CV [%]	1.42	1.54	0.816

Method comparison (n=100)	
Test x	Competitor bile acids (Hitachi 917)
Test y	DiaSys Total bile acids 21 FS (BioMajesty® JCA-BM6010/C)
Slope	1.02
Intercept	0.284 µmol/L
Coefficient of correlation	0.997

** according to CLSI document EP17-A2, Vol. 32, No. 8

Reference Range [20,21]

< 10 µmol/L (fasting)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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Additions and/or changes in the document are highlighted in grey. For deletions, please refer to the customer information for the corresponding edition number of the package inserts.

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