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Bile Acids, Enzymatic cycling

(en) English

REF	Content								
903100B	1 x	0.9	L	R1	+	3 x	0.1	L	R2
903110	4 x	90	mL	R1	+	1 x	120	mL	R2
903115	4 x	45	mL	R1	+	1 x	60	mL	R2
903120	4 x	22.5	mL	R1	+	1 x	30	mL	R2
903125	4 x	9	mL	R1	+	1 x	12	mL	R2
950911	4 x	45	mL	R1	+	3 x	20	mL	R2
90410917	3 x	60	mL	R1	+	1 x	60	mL	R2
9A0808	3 x	20	mL	R1	+	1 x	20	mL	R2
9T1008	3 x	20	mL	R1	+	1 x	20	mL	R2
9K0707	4 x	45	mL	R1	+	1 x	60	mL	R2
9E1808	2 x	37.5	mL	R1	+	2 x	12.5	mL	R2

For professional in vitro diagnostic use only.

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE[1,2]

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.

TEST PRINCIPLE

bile acids + Thio-NAD $\xleftrightarrow{3-\alpha-HSD}$ Oxid. bile acids + Thio-NADH

Oxid. bile acids + NADH $\xleftrightarrow{3-\alpha-HSD}$ bile acids + NAD

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 NADH to bile acids and NAD. The presence of excess NADH efficiently promotes the enzyme cycling and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405 nm.

Abbreviations:

TBA	=	Total Bile Acids
NAD	=	Nicotinamide Adenine Dinucleotide
NADH	=	reduced NAD
3-α-HSD	=	3-α-Hydroxysteroid dehydrogenase

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1	
Buffer	
Thio-NAD	> 0.1 mM
Reagent 2	
Buffer	
3-α-HSD	> 2 kU/L
NADH	> 0.1 mM

MATERIAL REQUIRED BUT NOT PROVIDED

Standard or Calibrator eg:

REF	Name	Content
903210	Bile Acids Standard	1 x 3 mL

Controls, eg:

REF	Name	Content	Description
D98481	Diacon N	12 x 5 mL	Control normal
D14481	Diacon N	5 x 5 mL	Control normal
D98481SV	Diacon N	1 x 5 mL	Control normal
D98482	Diacon P	12 x 5 mL	Control abnormal
D14482	Diacon P	5 x 5 mL	Control abnormal
D98482SV	Diacon P	1 x 5 mL	Control abnormal

- NaCl solution (9 g/L).
- Photometric device.
- General laboratory equipment.

REAGENT PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

Conditions:	Store at 2 – 8 °C. Protect from light! Close immediately after use.
Stability:	Unopened reagents are stable until the expiration date printed on the label.

The reagents are light sensitive. The intrinsic yellow to yellow-brown colour of the reagent does not interfere with the test.
Note: reagents from different lots must not be interchanged.

WARNINGS AND PRECAUTIONS

- Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures.
- Do not swallow! Avoid contact with skin and mucous membranes.
- Please refer to the safety data sheet 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.
- In the event of an incident related to the device, report it to the manufacturer and your competent authority as required.
- For professional use only!

SPECIMEN COLLECTION AND STORAGE[4]

Use fresh patient serum, EDTA treated plasma or Lithium heparin plasma samples. TBA concentration is increased after meals; hence, samples should be collected under fasting conditions*.

Stability:

Serum or plasma:	at 4 °C	1 week
	at - 20 °C	3 months

Discard contaminated specimens.

It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory[5].

*This does not apply to women with intrahepatic cholestasis of pregnancy who will need peak bile acid testing and samples should therefore be taken post-prandially.

STANDARD

(not included in the kit – has to be ordered separately)

Concentration	50 µmol/L
Storage:	2 – 8 °C
Stability:	up to the expiration date

Close immediately after use! Avoid contamination! Protect from light.

TEST PROCEDURE

Method:	Colorimetric, 2 Point Kinetic (fixed time), Increasing reaction, enzymatic cycling
Wavelength:	405 nm
Optical path	1 cm
Temperature:	37 °C

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Standard	Sample
Reagent 1	900 µL	900 µL	900 µL
Sample	-	-	14 µL
Standard	-	14 µL	-
Dist. water	14 µL		
Mix. Incubate for 3 – 5 minutes at 37°C, then add:			
Reagent 2	300 µL	300 µL	300 µL
Mix, incubate for 60 sec. at 37 °C and measure absorbance A1 at 405 nm. Incubate for another 60 sec. at 37°C and measure absorbance A2 at 405 nm. Calculate change in absorbance: ∆A= A2 – A1			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

$$\text{TBA [µmol/L]} = \frac{\Delta A \text{ Sample} - \Delta A \text{ Blank}}{\Delta A \text{ Std.} - \Delta A \text{ Blank}} \times \text{conc. Std [µmol/L]}$$

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. We recommend the DIALAB multi control sera **Diacon N** (with values in the normal range) and **Diacon P** (with values in the pathological range). Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration

The assay requires the use of a Bile Acid Standard or Calibrator. We recommend the Dialab **Bile Acids Standard**. Use 0.9% saline as zero calibrator.
Calibration frequency may vary and is dependent on instrument application.

PERFORMANCE CHARACTERISTICS

Accuracy and precision

The within-run precision and between-run precision were evaluated in samples containing two different bile acid levels (8 µM and 23 µM) in 20 runs.
CV ≤ 3.9 % for within-run precision and CV ≤ 2.9 % for between-run precision.

Analytical sensitivity

Lower limit of linearity is 1 µmol/L.

Linearity and measuring range

The test has been developed to determine bile acids concentrations within a measuring range from 1 – 180 µmol/L in serum/plasma.

Analytical specificity

No interference up to:

Ascorbic acid	50 mg/dL
Bilirubin	50 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	750 mg/dL

Clinical performance

A comparison between DIALAB Bile Acids, Enzymatic cycling (x) and a commercially available test (y) using 52 serum samples ranging from 0.47 – 131.25 µmol/L gave following results:
y = 1.1536 x – 0.8567 µmol/L; r = 0.992.

A matched set of 39 serum and lithium heparin plasma samples ranging from 0.14 - 21.18 µmol/L gave the following results:
Lithium heparin = 0.9972 (serum) + 0.1178 µmol/L; r = 0.9805

Tests were performed on the following instrument: Hitachi 717.

TRACEABILITY

The Bile Acids standard is traceable to the Sigma Diagnostics Bile Acids Calibrator.

EXPECTED VALUES[3]

In serum / plasma: 0 – 10 µmol/L
Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATIONS

- Samples with bile acid levels 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 this product.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. LaRusso, N.F. et al., Dynamics of Enterohepatic Circulation of Bile Acids, New Engl J M 1974; 291, 689-692.
2. Skrede S. et al: Bile acids measured in serum during fasting as a test for liver disease, Clin Chem 1978, 24: 1095-1099.
3. Wu, Alan H.B. Tietz Clinical Guide to Laboratory Tests. 4th ed. St. Louis, MO: Saunders/Elsevier, 2006. 170-171.
4. Ovadia C, Seed P, Sklavounos A, et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. Lancet 2019; 393(10174):899-909.
5. CLSI, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline, H18-A4, Vol.30 No. 10.

USED SYMBOLS

Symbol	Description
Cont.	Content

CE IVD 2°C 8°C