BILE ACIDS (LIQUID) REAGENTS (Colorimetric) MANUAL OR AUTOMATED APPLICATIONS C404 series

Intended Use
For **In Vitro Diagnostic** use in the automated, quantitative determination of Bile Acids in serum or plasma.

Clinical Significance (1-4)
Bile Acids are found in most body fluids and are present in highest concentrations in bile. The most important and more abundant Bile Acids are cholic acid, deoxycholic acid, lithocholic acid, and chenodeoxycholic acid. Conjugates of Bile Acids with glycine, taurine and in some cases with glucuronide or sulfate esters are secreted into the bile where they undergo enterohepatic circulation.
Increases of serum Bile Acids, measured in the fasting and postprandial state or by use of a tolerance test, reflect hepatic injury. Decreased levels indicate Bile Acid malabsorption, possibly due to ileal dysfunction.

Method Principle (5-7)
Several procedures are noted in the literature for measuring Bile Acids in serum. The Catachem Bile Acids procedure is based on the enzymatic procedure described by Mashige et al. In this Bile Acids procedure, 3α-hydroxy Bile Acids are converted to corresponding 3-keto hydroxy Bile Acids by the action of the enzyme 3α-hydroxysteroid dehydrogenase (3∝-HSDH) with concomitant reduction of NAD^+ to NADH. The NADH thus produced is subsequently oxidized to NAD^+ in a diaphorase-catalyzed reaction where nitrotetrazolium blue (NTB) is reduced to produce a formazan dye, which has an absorption maximum at 540 nm. The intensity of the color produced in a rate reaction is directly proportional to the concentration of Bile Acids in the serum sample. The reaction scheme below illustrates the reactions that take place in this Bile Acids procedure.

$$\text{1.) 3}\alpha\text{-hydroxy bile acids} + \text{NAD} \xrightarrow{3\alpha\text{-HSDH}} \text{3keto-hydroxy Bile Acids} + \text{NADH}$$

$$\text{2.) NADH + NBT} \xrightarrow{\text{Diaphorase}} \text{NAD + Formazan}$$

REAGENTS
Bile Acids Enzyme Reagent (R1)
Each liter contains:

Buffer	
3α-hydroxysteroid dehydrogenase	≥800 Units/L
Diaphorase	≥5000 Units/L
Stabilizer and nonreactive ingredients	

Bile Acids Activator Reagent (R2)

NAD	6.0 mmol
NBT	0.6 mmol

Stabilizer and nonreactive ingredients

Precautions
Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage And Stability
Store the Bile Acids Enzyme Color Reagent (R1) and Activator Reagent (R2) at 2-8°C. When stored as directed these reagents are stable until the expiration date stated on the label.

Working Reagent Preparation
The Bile Acids Enzyme Color Reagent and Activator Reagent are packaged in ready-to-use form. No preparation is required. Once in use, label reagents "Working Reagent R1" and "Working Reagent R2" respectively. Store the Working Reagents at 2-8°C. When stored as directed the Working Reagents R1 and R2 are stable for 60 days at 2-8°C.

Reagent Indications Of Deterioration
- Turbidity
- Quality control values out of assigned ranges.

If these reagent characteristics are observed, contact your technical representative.

Specimen Collection And Stability
Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. Bile Acids in serum are stable 7 days at room temperature, 10 days refrigerated at 2-8°C and for many months frozen at -20°C. (2)

Procedure
These instructions are outlined for performing the Bile Acids assay using a manual procedure.

Materials Provided
Catachem Bile Acid Reagents:
Catachem Bile Acids Enzyme Color Reagent (R1)
Catachem Bile Acids Activator Reagent (R2)

Materials Required But Not Provided
- Spectrophotometer
- Catachem Bile Acids Calibrator material with assigned Bile Acid values
- Catachem Bile Acids Quality Control material with assigned Bile Acid values

Calibration
Catachem Bile Acids Calibrator which contains a known Bile Acids value is recommended.

Directions For Use
The Catachem Bile Acids method requires two reagents, R1 and R2.

Quality Control
To monitor the quality performance of the procedure used, Catachem Bile Acids Control Level I and Control Level II with assigned Bile Acids values should be included in the assay each time the procedure is run.

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Assay Procedure

1. Label cuvettes or appropriate test tubes as: a) Calibrator blank (CAL-BLK), b) Calibrator (CAL), c) Control 1 blank (C-1BLK), d) Control 1 (C1), e) Control 2 blank (C-2BLK), f) Control 2 (C2), g) Sample blank (SAMP BLK), h) Sample (SAMP).
2. Pipette the reagent and sample volumes into the cuvettes or test tubes as shown in table below. Pipette Bile Acids Enzyme Reagent I (R-1) first, followed by the sample or water.
3. Incubate for 5 minutes at 37°C to allow clearing of lipemic samples.
4. Immediately, after the incubation period is over, add Bile Acids Activator Reagent II (R-2) and mix all cuvettes at 37°C.
5. Set a timer for exactly 5 minutes at 37°C.
6. At the end of the 4 minutes read all cuvettes at 540nm. Record all absorbencies.

	CAL BLK	CAL	C-1 BLK	C-1	C-2 BLK	C-2	SAMP BLK	SAMP
	ml	ml	ml	ml	ml	ml	ml	ml
RGT 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SAMP	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
H_2O	0.05	0.0	0.05	0.00	0.05	0.00	0.05	0.00
INCUBATE FOR 5 MINUTES AT 37°C								
RG2 2	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
INCUBATE FOR 4 MINUTES								
MIX AND READ ALL CUVETTES AT 37°C								

Calculations and Results

$$\text{Bile Acids } \mu\text{mol/L} = \frac{\Delta\text{- Abs. Samp.}}{\Delta\text{- Abs. Cal.}} \times \text{Cal. } \mu\text{mol/L}$$

Example:

	Samp. Abs.	Blk. Abs.	Δ-Abs.
Sample	0.400	0.300	0.100
Calibrator	0.150	0.030	0.120

Calibrator assigned value = 100 µmol

$$\text{Samp Bile Acids } \mu\text{mol/L} = \frac{0.100}{0.120} \times 100 \ \mu\text{mol/L}$$

$$= 83 \ \mu\text{mol/L}$$

Interfering Substances

Samples with the following concentration substances have no significant effect on the accuracy of this Bile Acids procedure:

- Lipemia (triglycerides) ≤ 1000 mg/dL
- Extremely icteric serum may produce erroneous results. If this is the case, make a suitable sample dilution with physiological saline and assayed sample. Multiply the result obtained by the dilution factor.

Other substances and certain drugs are also known to influence the Bile Acids values. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young et al (8).

Procedure Limitations

Samples with Bile Acid values greater than 250 µmol/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Method Performance Characteristics

Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.0016-0.0024 per mg/ml should be obtained. Lowest Limit of Detection (LLD) was determined to be 15 µmol/L.

Linearity: In this procedure there is not significant nonlinearity over the range of 15-250 µmol/L.

Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure (9). The following results were observed.

Precision

BILE ACIDS	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
µmol/L	µmol/L	%	µmol/L	%
25	0.77	3.0	1.23	5.01
150	2.84	1.86	7.30	5.08
250	2.21	0.88	11.67	4.56

ACCURACY

Correlation studies were carried out between this automated Bile Acids method (Y) and a reference automated Bile Acids procedure based on the 3∝-HSDH and Diaphorase reactions (X). Canine serum samples were assayed and the results compared by the least square regression. The following statistics were observed:

N	=	12
Range	=	4.1-171
Mean Y	=	41.88
Mean X	=	43.84
Y	=	0.959x – 0.16
Sy.x	=	11.29
r	=	0.978

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