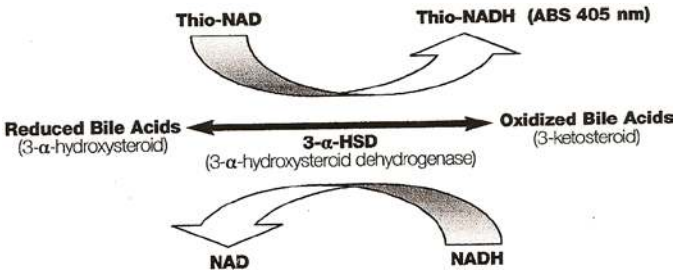
VetSpec™ *BILE ACIDS II*
ENZYME CYCLING METHOD
Manual spectrophotometric application
C562 Series and V562 Series

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 in 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 (5-6)
Several procedures have been reported in the literature for measuring Bile Acids in serum. Catachem Bile Acids procedure is based on the enzyme cycling procedure described by Komiyama et al. In this Bile Acids procedure, 3-α-hydroxy Bile Acids are converted to their corresponding 3-keto hydroxy Bile Acids by the action of the enzyme 3-α-hydroxysteroid dehydrogenase (3-∝-HSDH) with concomitant reduction of Thio-NAD to Thio-NADH. The presence of Free NADH accelerates enzyme cycling thus producing a strong absorbance signal due to the efficient formation of Thio-NADH. The intensity of the absorbance produced 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.



REAGENTS

Bile Acids Cofactor Color Reagent (R1)
Buffer
Thio-NAD 3.5 mmol/L
Stabilizer and nonreactive ingredients.

Bile Acids Activator Reagent (R2)
Buffer
3-α-hydroxysteroid dehydrogenase ≥7000 Units/L
NADH 7.2 mmol/L
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 Cofactor Color Reagent (R1) and Activator Reagent (R2) at 2-8°C. When stored as directed these reagents are stable until expiration date stated on the label.

Working Reagent Preparation
The Bile Acids Enzyme Cofactor Color Reagent and the Bile Acids Activator Reagent are packaged in ready-to-use form. No preparation is required. Label these reagents "Working Reagent R1" and "Working Reagent R2" respectively. Store these Reagents, once opened at 2-8°C. When stored like this and capped when not in use reagents are stable for at least 60 days.

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

If these reagent characteristics are observed call your technical representative.

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

Procedure
These instructions are outlined for performing the Bile Acids assay using an automated procedure on the Beckman AU line of analyzers.

Materials Provided
Bile Acid Reagents:
Bile Acids Cofactor Reagent (R1)
Bile Acids Activator Reagent (R2)

Materials Required But Not Provided
- Automatic analyzer.
- Calibrator material with assigned Bile Acid values.
- Quality control material with assigned Bile Acid values.

Calibration
Catachem Bile Acids Calibrator, C404-05, C404-15 or V404-23 which contains a known Bile Acids value, is recommended.

The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the area in which it is located.

Quality Control
To monitor the quality performance of the procedure used, Catachem's Bile Acids Control I (C04-06, C404-16 or V404-24) and Control II (C404-07, C404-17 or V404-25) with assigned Bile Acids values are best included in the assay procedure.

Directions For Use
Catachem's Bile Acids method requires two reagents; R1 and R2 Reagents are packaged in ready-to-use form. No preparation is required.

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

Reagents are packaged in ready-to-use form. No preparation is required.

Procedure (Manual spectrophotometer)
Method is a Rate Method not an Endpoint so accuracy of read times is important.

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. Starting a timer, immediately, after the incubation period is over, add Bile Acids Activator Reagent II (R-2) at 15 second intervals to all cuvettes and mix.
5. Read OD's at 405 nm of all cuvettes at 37C after their 5 minute incubation period with R2 using the same 15 second interval between samples.
6. At the end of the 5 minutes read all cuvettes at 405 nm. 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 each sample/reagent mix FOR 5 MINUTES								
Read cuvettes at 37°C after their 5 Min incubation period								

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 (as example) = 100 µmol

$$\text{Samp Bile Acids } \mu\text{mol/L} = \frac{0.100}{0.120} \times 100 \ \mu\text{mol/L}$$
$$= 83 \ \mu\text{mol/L}$$

Canine Target Values:

Minimum:	0.0 µmol/L
Maximum:	8.0 µmol/L

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

Ascorbic Acid	≤ 50 mg/dL
Bilirubin	≤ 30 mg/dL
Hemoglobin	≤ 600 mg/dL
Lipemia (Triglycerides)	≤ 1000 mg/dL

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 (7).

Procedure Limitations
Samples with Bile Acids values greater than 200 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 0.007-0.009 per /min/µmol of Bile Acids should be obtained.
Limit of Quantitation: 1.7 µmol/L
Linearity: In this procedure there is no significant nonlinearity over the range of 0-200 µmol/L.
Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure (8). The following results were observed.

Precision

BILE ACIDS	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
µmol/L	µmol/L	%	µmol/L	%
7.80	0.524	6.696	0.534	5.734
22.90	0.550	2.401	0.596	2.326
55.00	1.600	2.911	0.994	1.753

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 Enzyme Cycling reaction (X). Human serum samples were assayed and the results compared by the least square regression. The following statistics were observed:

N	=	86
Range	=	1.1-166.8
Mean Y	=	67.16
Mean X	=	72.84
Y	=	1.030x – 3.96
Sy.x	=	6.79
r	=	0.9914

Bibliography

1. Demers L.M.: Bile Acids in Methods of Hormone Radioimmunoassay, 2nd ed. B.M. Jaffe, H.R. Behman, Editors. Academy Press, New York, 1979.
2. Heubi J.E., Balistreri WF, Partin J.C., Schubert W.K., Suchy F.J.: Enterohepatic circulation of bile acids in infants and children with ileal resection. J Lab Clin Med 95:231, 1980.
3. Roda A., Kricka L.A., DeLuca M., Hofman A.F.: Bioluminescence measurement of primary bile acids using immobilized 7 ∝-hydroxy-steroid dehydrogenase: Application to serum bile acids. J Lipid Res.23:1354, 1982.
4. Gilmore I.T., Hofman A.F, Altered drug metabolism and elevated serum bile acids in liver disease: A unified pharmacokinetic explanation. Gastroenterology 78:177, 1980.
5. Mashige F., Imai K., Osuga T.: Clin Chim Acta 70 (1976) 79-85).
6. Komiyama Y.I, Adachi, T., Ito, Y., Hikano, K., Sugiura, M., Sawki, S. Microassay of Serum Bile Acids By Enzymatic Cycling Method, Chem Pharm Bull (Tokyo) 30: 3796-3797 (1982).
7. Young D.S., Pestamer L.C., Gibberman V.: Effects of drugs on clinical laboratory tests. Clin Chem 21 No. 5(1975).
8. Evaluation of Precision Performance of Clinical Chemistry Devices, Second Edition. NCCLS Document EP5-T2, Vol. 12, No. 4.

Rev: AF190815dt