

Conjugated Bile Acids RIA

Radioimmunoassay for the quantitative determination of Bile Acids
in human serum and plasma.

REF

BD13011

CONJUGATED BILE ACIDS COMPONENT SYSTEM

For the Quantitative Determination of Conjugated Bile Acids in Serum or Plasma.

Summary and Explanation of the Test

Bile acids are C_{24} steroids derived from cholesterol. Cholic and cheno-deoxycholic acids are the two primary bile acids in man. In bile, they are conjugated with the amino acids, glycine and taurine, forming the four conjugated bile acids which are measured by this assay: glycocholic acid, taurocholic acid, glycochenode-oxycholic acid and taurochenodeoxy-cholic acid.

The primary bile acids undergo bacterial degradation in the intestine to form the secondary bile acids, deoxycholic, lithocholic acids and oxidation products.

The salts of the conjugated bile acids are powerful detergents which aid in intestinal absorption of lipids. They are almost entirely absorbed by the intestine and returned to the liver and gall bladder via the enterohepatic circulation. Normally, less than 1% of the bile acid pool is found in systemic blood.

It has been known for many years that the concentration of bile acids in serum is increased in patients with liver disease as a result of the failure of the diseased liver to extract bile acids efficiently from portal blood¹. Serum conjugated bile acid concentrations have been reported to be raised in many forms of structural liver disease.

Metabolic hepatic diseases involving organic anions do not seem to cause abnormal bile acid concentrations². No increase in serum conjugated bile acids, even in the presence of liver disease, will be noted in patients with intestinal malabsorption such as is found following ileal resection or by-pass^{2,3}. It has been suggested that bile acid levels may be the single most sensitive and specific indicator of liver disease where other chemical and enzyme tests are often normal^{2,4-9}, suggesting application for following recovery or predicting relapse in diseases such as hepatitis and for monitoring drug toxicity.

Principle of the Test

In radioimmunoassay, the antibody used should have an equal affinity for the standard and the analyte which is present in the patient's serum or plasma. The unlabeled analyte competes with labeled analyte for the limited number of available antibody binding sites thereby reducing the amount of labeled analyte bound to antibody. The

level of radioactivity bound is therefore inversely related to the concentration of analyte in the patient sample or standard.

In the ICN Pharmaceuticals procedure, solid phase antibody-coated tubes and an [¹²⁵I] tracer are used for the measurement of conjugated bile acids in small volumes of serum.

Reagents

For *In Vitro* Diagnostic Use

1. **Bile Acids Solid Phase Tubes**, Catalog No. 06B243011. Contains 100 polystyrene tubes coated with antiserum (rabbit).

Storage: Refrigerate at 2-8°C tightly sealed in the original package. Warm to room temperature before opening. DO NOT INTERMIX DIFFERENT LOTS OF TUBES WITHIN ONE ASSAY. **Stability:** Refer to expiration date on bag.

2. **Bile Acids Standards Set**, Catalog No. 06B262056, containing sodium taurocholate in buffer with human serum albumin, ** 0.5% sodium azide* and other preservatives. Minimum volume 2.0 mL/vial for Standard A; 1.5 mL/vial for Standards B-F. Ready to use. **Storage:** Refrigerate at 2-8°C. **Stability:** Refer to expiration date on vial.

Standard	Standard Level $\mu\text{mole/L}$
A	0
B	0.4
C	2.0
D	6.0
E	15
F	50

3. **Bile Acids Tracer [¹²⁵I]**, Catalog No. 06B229717, containing glycocho-lic acid derivative [¹²⁵I] in buffer with bovine gamma globulin, 0.1% sodium azide*, preservatives and dye. The bottle contains < 5 μCi (185 kBq) of [¹²⁵I]; 110 mL/bottle. Ready to use. **Storage:** Refrigerate at 2-8°C. **Stability:** Refer to expiration date on vial.

* **WARNING:** Reagents contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metallic azides. Very toxic if swallowed. Contact with acids liberates very toxic gas. After contact with skin,

wash immediately with plenty of water. On disposal, flush with a large volume of water to prevent azide build-up.

**** CAUTION:** Handle as if capable of transmitting infection: Source material from which this product was derived was found non-reactive for HBsAg and negative for HIV antibody when tested with licensed reagents. No known test method can offer assurance that product derived from human blood will not be infectious. Refer to CDC/NIH Biosafety in Micro-biological and Biomedical Laboratories publication (HHS Publication No. [CDC] 84-8395).

**WARNING: CONTAINS
RADIOACTIVE MATERIAL**

This ICN Pharmaceuticals Solid Phase Component System contains < 5 microcuries (185 kilobecquerels) of [¹²⁵I]. This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

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Adherence to the basic rules of radiation safety should provide adequate protection. The user is referred to National Bureau of Standards Handbook No. 92, "Safe Handling of Radioactive Materials", issued March 9, 1964, Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. A summary follows:

- ◆ Do not eat, drink, smoke or apply cosmetics where radioactive materials are used.
- ◆ Do not pipet radioactive solutions by mouth. ◆ Avoid direct contact with all radioactive materials by using protective articles such as lab coats and disposable gloves. ◆ All radiological work should be done in a designated area away from traffic.
- ◆ Radioactive materials should be stored in their original containers in a designated area. ◆ A record book for logging receipt and disposal of all radioactive materials should be kept. ◆ Laboratory equipment and glass-ware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes. ◆ Any radioactive spills should be taken care of immediately in accordance with established procedures. ◆ All radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory. ◆ Uncontaminated containers may be discarded in non-radioactive waste providing that labels and labeling are defaced.

Equipment and Reagents Required but Not Provided in Kit:

1. Evacuated glass tube (containing EDTA or heparin if desired).
2. Distilled water.
3. Test tube rack.
4. Automatic pipettor-dilutor or semi-automatic pipettes capable of sampling 25 μL and delivering 1000 μL .
5. Water bath capable of maintaining $37 \pm 1^\circ\text{C}$.
6. Aspirator
7. Syringe with Tip, 2 mL, and Metal Holder, and filling outfit. Disposable Needle Tubing Adapter, 18 gauge.
8. Gamma counter for measuring [^{125}I].
9. Suitable graph paper such as linear, semi-log or logit-log paper.

Specimen Collection

The specimen should be identified as fasting or 2 hour postprandial. Standard meals have been used in some studies¹¹. **Handle all blood and serum as infectious materials.**

1. **Preparation of Specimen for Analysis:** Collect the blood in a 5 or 10 mL evacuated glass tube. Use heparin or EDTA if plasma is desired for analysis. If serum is being collected, allow the blood to clot at room temperature. Centrifuge and collect the serum or plasma.
2. **Storage:** Store the serum or plasma before analysis at 2-8°C. If storage is expected to exceed 48 hours, the sample should be stored at -20°C or below and thawed once only. Repeated freezing and thawing or long storage unfrozen can result in cloudy sera; such deteriorated samples may give erroneous results.
3. **Shipping of Specimens:** Carefully packed serum must be shipped at 2-8°C. If shipping time is expected to exceed 48 hours, the sample should be shipped frozen.

Assay Procedure

Coated tubes, reagents and samples must be brought to room temperature before use, but in order to minimize deterioration return to recommended storage immediately after use. Do not use reagents other than those provided as a matched set. **OPTIMAL RESULTS WILL BE OBTAINED BY STRICT ADHERENCE TO THIS PROTOCOL. CAREFUL PIPETTING IS ESSENTIAL.**

In the following protocol, assay of the standard level points in duplicate is recommended. Patient samples must be assayed in duplicate and the preparation of the standard curve and the clinical determinations must be run simultaneously. Control sera should be run concurrently with patient samples.

The assay uses 25 μL of undiluted patient sample. Samples with values $> 50 \mu\text{mole/L}$ may be diluted for reassay. If a serum appears jaundiced or if the patient is known to have high values of conjugated bile acids, a 1:8 or higher dilution may be used.

1. Number 12 Bile Acids Solid Phase Tubes for the standard curve. Beginning with 13, number two Solid Phase Tubes for each patient sample.
2. Add 25 μL Bile Acids Standards and patient samples according to the outline which follows.
3. Add 1.0 mL Bile Acids Tracer [^{125}I] Solution to all tubes.* Do not vortex these tubes.
4. Incubate all tubes in a water bath at $37 \pm 1^\circ\text{C}$ for 60 minutes from the time of the last addition in the previous step. Place the entire rack of tubes in the bath at once. Do **not** use an oven or other air heating device.
5. Remove the rack of tubes from the bath and aspirate or decant all fluid. Wash with 1.0 mL distilled water and aspirate or decant again.
6. Obtain a background count for the counter. Count the radioactive tubes in sequence for 0.5-2 minutes with a gamma counter. Tubes 1 and 2 will give the Trace Level count. The counts in these tubes should be 7,000-30,000 cpm. The efficiency of the gamma counter will determine the counting time. The counting time required for an accumulated Trace Level count of 10,000 will indicate the time required for all assays with the user's gamma counter.

* If an automatic sampling/ dispensing device is used to combine steps 2 and 3, a tip of sufficient diameter should be selected so that no excessive turbulence or bubble formation occurs. Recommended tips are 1.0 mm for the Micromedic and 1.5 mm for the Cordis dispensers. The tip is allowed to touch the inside of the test tube at the approximate midpoint during the dispensing step.

CONJUGATED BILE ACIDS SOLID PHASE RADIOIMMUNOASSAY

Tube No.	Standard (μL)	Patient Serum (μL)	Tracer	Incubate	Wash
1, 2	25 A	---	Add 1000 μL to all tubes	Incubate all tubes at 37 $\pm 1^\circ\text{C}$ for 60 minutes	Aspirate or decant and wash all tubes
3, 4	25 B	---			
5, 6	25 C	---			
7, 8	25 D	---			
9,10	25 E	---			
11,12	25 F	---			
Patient Samples		25			

Count the radioactivity in all tubes

Calculation of Results

Logit-Log Calculations.

1. Determine the background count of the gamma counter.
2. Subtract the background from all tube counts to obtain the corrected counts per minute (or per uniform time). Use only the corrected counts in these calculations.
3. Average the corrected counts for tubes 1 and 2 to give the average Trace Level Count.
4. Divide the corrected counts for each tube by the average Trace Level Count to give the % of Trace Level for each tube. A separate value for each duplicate should be obtained.
5. A Standard Curve may be plotted as follows:
Using logit-log paper, plot % of Trace Level versus $\mu\text{mole/L}$ Conjugated Bile Acids Standard on the log scale. Counting data and calculated % of Trace Level are in Table 1 (Page 38). The standard curve plotted for these data is shown in Figure 1 (Page 39).
6. Determine the conjugated bile acids concentrations in the patient samples from the calculated % of Trace Level by interpolation from the standard curve.
7. Calculate diluted patient samples as follows: $\mu\text{mole/L}$ in diluted patient sample (from standard curve) \times dilution factor = $\mu\text{mole/L}$ conjugated bile acids in undiluted patient serum.
8. Sample Calculation for a Patient Specimen:

$$\begin{array}{rcl} \text{Count (found)} & = & 5663 \\ \text{Background} & = & 288 \\ \text{\% of Trace Level} & = & \frac{5663 - 288}{9772} \times 100 = 55.0\% \end{array}$$

The logit-log standard curve of % of Trace Level (Figure 1) shows that 55.0% corresponds to a conjugated bile acids concentration of 1.9 $\mu\text{mole/L}$ in the patient sample.

The average of this value and the value for the duplicate determination is reported as the Conjugated Bile Acids concentration in $\mu\text{mole/L}$.

Limitations of the Procedure

The ICN Pharmaceuticals Conjugated Bile Acids Solid Phase Component System can

be used to measure the concentration of the major conjugated bile acids which are elevated in the majority of pathological liver conditions. The minor conjugated bile acids and the unconjugated bile acids have minimal effect on the results due to their relatively lower serum concentrations and/or lower cross reactivity with the antibody.

Despite the variability of the cross reactivity of the conjugated serum bile acids, this system yields satisfactory correlations with the 3 α -hydroxy-steroid dehydrogenase assay which is known to measure total serum bile salt levels.

Expected Values

Serum values of healthy volunteers (fasting) were:

$$\begin{aligned} N &= 162 \\ \text{Mean} &= 2.23 \mu\text{mole/L} \\ \text{S.D} &= 1.95 \mu\text{mole/L} \end{aligned}$$

Typical serum levels obtained with this system for specific liver diseases are graphically depicted in Figure 2.

Use of standard meals for 2 hour postprandial serum bile acids determination may be of value in standard-izing the methodology¹¹.

The following ranges of expected values obtained with this system can be used as a guideline:

Normal - less than 6 $\mu\text{mole/L}$
Abnormal - greater than 6 $\mu\text{mole/L}$

Specific Performance Characteristics

Accuracy:

1. A linear regression analysis comparing the results obtained for 45 patient sera using this product with those obtained at a university hospital by the 3 α -hydroxysteroid dehydrogenase method gave the following results:

$$\begin{aligned} \text{Correlation coefficient,} &= 0.94 \\ \text{Slope,} &= 1.18 \\ \text{Y-intercept,} &= -6.4 \end{aligned}$$

2. Recovery was done by adding one volume of a glycocholic acid and taurocholic acid mixture (3:1) to various volumes of a low serum pool.

Bile Acids Added $\mu\text{mole/L}$	Bile Acids Corrected Found $\mu\text{mole/L}$	% Recovery
20.9	17.6	84.2
10.5	9.3	88.6
5.2	5.2	100
3.8	3.4	89.5

Precision:

Human sera and a commercial control representing normal and high values were assayed to determine inter-assay and intra-assay variations.

	Inter-Assay			Intra-Assay		
	1	2	3	1	2	3
Sample No. of Determinations	25	19	25	20	20	20
Mean, $\mu\text{mole/L}$	3.2	6.9	13.2	3.8	6.8	11.4
S.D.	0.6	1.0	1.9	0.5	0.7	0.7
% CV	18	14	14	12	11	7

Sensitivity:

0.2 $\mu\text{mole/L}$ as determined by the concentration of taurocholic acid at 90% of trace binding.

Specificity:

Analyte	% Cross-Reactivity (Weight basis)
1. Conjugated Bile Acids	
Taurocholic acid	100.0
Glycholic acid (in serum)	85
Taurochenodeoxycholic acid	210
Glycochenodeoxycholic acid	62
Taurodeoxycholic acid	4.0
Glycolithocholic acid	3.3
Glycodeoxycholic acid	3.3
2. Unconjugated Bile Acids	
Chenodeoxycholic acid	8.1
Cholic acid	3.1
Deoxycholic acid	0.2
Lithocholic acid	0.7
Dehydrocholic acid	0.03
3. Steroids (at 10x normal physiological levels)	N.D.*
Cortisol	N.D.
Aldosterone	N.D.
Estriol	N.D.
Testosterone	
4. Miscellaneous (at specified levels)	N.D.
Cholesterol (1 mg/mL)	< 1
Ethanol (1.5 mg/mL)	N.D.
Digoxin (20 ng/mL)	N.D.
Spirolactone (20 ng/mL)	< 1
Heparin (1 mg/mL)	

*No detectable effect on B₀

TABLE 1
Conjugated Bile Acids Radioimmunoassay

Tube No.	Counts per Minute	Corrected Counts A	Average Counts B	% of Trace Level $\frac{A \times 100}{B}$	Standard Curve Concentration $\mu\text{mole/L}$
Background	288				---
	1005	9762	9772	---	0
Trace Level	1 0	9782		---	0
	2 1007	7737		79.2	0.4
	3 0	7964		81.5	0.4
	4 8025	5425		55.5	2.0
	5 8252	5648		57.8	2.0
	6 5713	3238		33.1	6.0
	7 5936	3211		32.8	6.0
	8 3526	1845		18.9	15
	9 3499	1714		17.5	15
	10 2133	919		9.4	50
	11 2002	984		10.1	50
	12 1207	5375		55.0	1.9
Patient Sample	1272				
	5663				

FIGURE 1
Typical Standard Curve with Logit Log Transformation

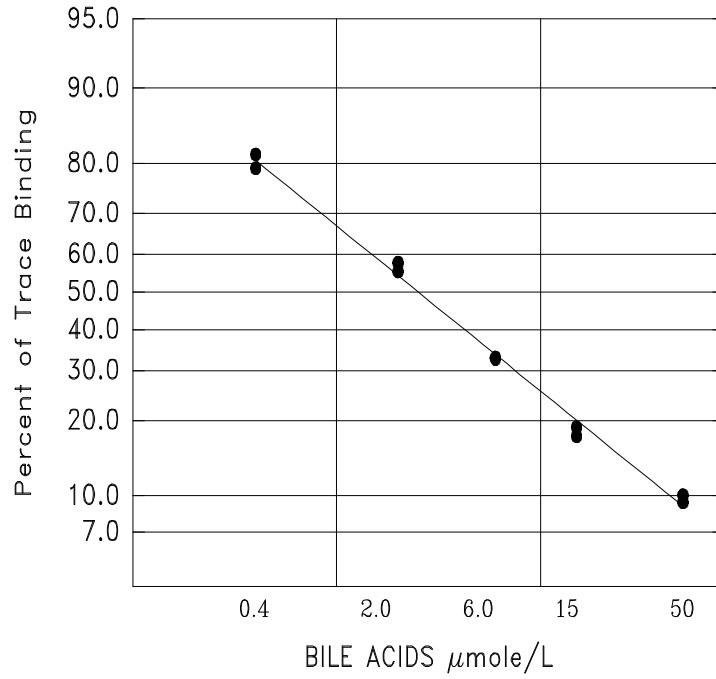
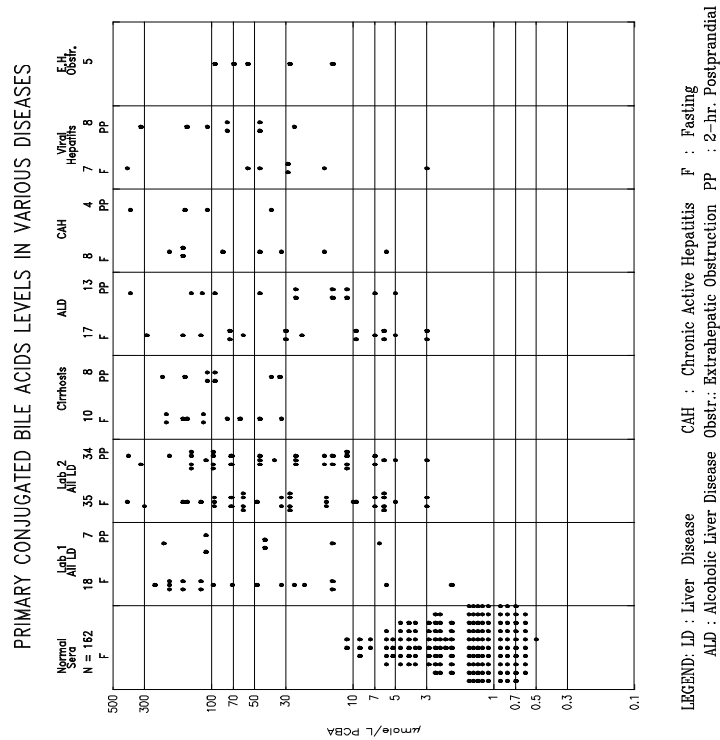


FIGURE 2



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