**Total Bile Acids**

**OSR61208**

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 28 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intended Use**

The Beckman Coulter Total Bile Acids reagent OSR61208 is intended for the *in vitro* quantitative determination of Total Bile Acids in serum or plasma on Beckman Coulter AU® clinical chemistry analyzers.

**NOTE:** For Veterinary Use Only

**Summary**

Total 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.

**Methodology**

The reagents of the assay kit are in a stable liquid formulation that allows for ease of use coupled with enhanced performance characteristics. 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 Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 410 nm.

**Reaction Principle**

\[
\begin{align*}
\text{Thio-NAD} & \quad \text{Thio-NADH} \\
\text{Bile acids} & \quad \text{Oxidized bile acids} \\
\text{3-α-HSD} & \quad \text{NAD} \quad \text{NADH}
\end{align*}
\]

**System Information**

For AU400/400e/480, AU600/640/640e/680, and AU2700/5400 Beckman Coulter Clinical Chemistry Analyzers.

**Reagents**

**Composition of Reactive Ingredients**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Thio-NAD &gt;0.1mM, Buffer</td>
</tr>
<tr>
<td>R2</td>
<td>3-α-HSD &gt;2kU/L, NADH &gt;0.1 mM, Buffer</td>
</tr>
<tr>
<td>Calibrator</td>
<td>Conjugated cholic acids, Buffer</td>
</tr>
</tbody>
</table>

**Precautions**

For *in vitro* diagnostic use.

Do not ingest. Harmful if swallowed.

To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent and calibrator.

Dispose of all waste material in accordance with local guidelines.

**Preparation of Reagents**

The Beckman Coulter Total Bile Acids reagents are ready for use. No preparation is required.

**Storage and Stability**

The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 8°C. Opened bottles of reagents are stable for 30 days when stored in the refrigerated compartment of the Beckman Coulter analyzers.

**Indications of Deterioration**

Visible signs of microbial growth, gross turbidity, precipitate or change in color in the TBA reagent may indicate degradation and warrant discontinuation of use.
Total Bile Acids

Specimen Collection and Preparation
Use fresh patient serum, EDTA treated or Lithium heparin treated plasma samples. Samples should be collected under fasting conditions. Specimens from patients, who are on Ursodeoxycholic Acids (UDCA) treatment, are not suitable for use with this Total Bile Acids Reagent.

Sample Storage and Stability
Serum or plasma samples are stable for a week at 4 °C, or for 3 months at –20 °C.

Interfering Substances
Results of studies show that the following substances do not interfere with this Total Bile Acids procedure. The criteria for no significant interference is recovery within 10% of the initial value.

- Bilirubin: No significant interference up to 12 mg/dL Bilirubin
- Hemolysis: No significant interference up to 300 mg/dL Hemolysate
- Lipemia: No significant interference up to 800 mg/dL Intralipid

* Intralipid, manufactured by KabiVitrum Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter, Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young\(^3\) for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User’s Guide appropriate to the analyzer.

Materials Provided
Beckman Coulter Total Bile Acids OSR61208

Materials Required But Not Provided
Beckman Coulter Total Bile Acids Calibrator ODR20070
Beckman Coulter Total Bile Acids Control ODC00036

Stability of Final Reaction Mixture
The Beckman Coulter analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration for the Total Bile Acids procedure is every 7 days. Calibration of this procedure is accomplished by use of the Beckman Coulter Total Bile Acids Calibrator ODR20070.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU clinical chemistry analyzer at least three levels of an appropriate quality control material should be tested a minimum of once a day. In addition, these controls should be tested after calibration with each new lot of reagent and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure.

Results
Automatically printed out for each sample in μmol/L at 37°C.

Dynamic Range
The Beckman Coulter TBA procedure is linear from 4 to 120 μmol/L. Samples exceeding the upper limit of linearity should be diluted with saline and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values
Serum or plasma containing 0-10 μmol/L bile acids is considered normal range for a fasting canine and 0-20 μmol/L for a post prandial canine. We suggest that each laboratory establish a normal range first before analyzing patient sample sets.

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the Beckman Coulter Total Bile Acids reagent on Beckman Coulter AU clinical chemistry analyzers according to established procedures\(^4\). Results obtained in individual laboratories may differ.
Total Bile Acids

Precision
Estimates of precision on the AU5400, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 6% and total precision is less than 8%. Assays of control material were performed and data reduced following CLSI guidelines.

<table>
<thead>
<tr>
<th>N= 80</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, (\mu)mol/L</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>10.9</td>
<td>0.174</td>
<td>1.6%</td>
</tr>
<tr>
<td>31.5</td>
<td>0.400</td>
<td>1.3%</td>
</tr>
<tr>
<td>112.6</td>
<td>1.23</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

Method Comparison
Canine serum samples were used to compare this Beckman Coulter Total Bile Acids Reagent on the AU5400 with another commercially available Total Bile Acids method. The table below demonstrates representative performance on AU clinical chemistry analyzers.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>AU5400</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Method</td>
<td>Method 2</td>
</tr>
<tr>
<td>Slope</td>
<td>1.132</td>
</tr>
<tr>
<td>Intercept ((\mu)mol/L)</td>
<td>-2.54</td>
</tr>
<tr>
<td>Correlation Coeff. (r)</td>
<td>0.9978</td>
</tr>
<tr>
<td>No. of Samples (n)</td>
<td>23</td>
</tr>
<tr>
<td>Range ((\mu)mol/L)</td>
<td>6.2 to 107.7</td>
</tr>
</tbody>
</table>

Sensitivity: Limit of Quantification (LOQ)
The Limit of Quantification (LOQ) using serum settings for the Beckman Coulter Total Bile Acids reagent was determined to be 3.0 \(\mu\)mol/L. This was determined according to CLSI protocol EP17-A and represents the lowest concentration of Total Bile Acids that can be measured with a total imprecision of 20%.

References
1. Clinical value of bile salt tests in anicteric liver disease
4. Data is on file for specific AU clinical chemistry analyzers.

Made in USA for: Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 1-800-223-0125