Total Bile Acids Assay Kit

Configuration
The Diazyme Total Bile Acids reagent is provided in bulk and the following kit configurations:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Catalog No.</th>
<th>Kit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal</td>
<td>DZ042A-K</td>
<td>R1: 2 x 60 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R2: 2 x 20 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cal: 1 x 2 mL</td>
</tr>
<tr>
<td>Beckman CX/LX</td>
<td>DZ042A-KB1</td>
<td>R1: 2 x 60 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R2: 2 x 20 mL</td>
</tr>
<tr>
<td>Olympus AU400</td>
<td>DZ042A-KY1</td>
<td>Cal: DZ042A-CAL*</td>
</tr>
<tr>
<td>Hitachi 917</td>
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<td></td>
</tr>
</tbody>
</table>

* Note: Calibrators Sold Separately

Intended Use
Diazyme Total Bile Acids Assay Kit is intended for the in vitro quantitative determination of serum total bile acids (TBA).

Clinical Significance 1,2
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.

Assay Principle
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 405 nm.

Materials Required But Not Provided
An analyzer capable of dispensing two reagents and of measuring absorbance at 405 nm with temperature control (37°C).

Reagent Composition

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Thio-NAD, Buffer</td>
</tr>
<tr>
<td>R2</td>
<td>3-α-HSD, Buffer</td>
</tr>
<tr>
<td>Calibrator</td>
<td>Glycolic acid, Buffer</td>
</tr>
</tbody>
</table>

Reagent Preparation
Diazyme Total Bile Acids Assay Reagents are ready-to-use, liquid reagents.

Reagent Stability and Storage
Unopened reagents are stable until the expiration date printed on the label. Reagents are light sensitive and should be stored at 2-8°C. Reagents from different lots must not be interchanged.

Specimen Collection and Handling
Use fresh patient serum, or EDTA treated or Lithium heparin treated plasma samples. TBA concentration is increased after meals; hence, samples should be collected under fasting conditions. Serum or plasma samples are stable for a week at 4 °C, or for 3 months at –20 °C.

Precautions
1. For in vitro diagnostic use.
2. Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid swallowing and contact with skin or mucous membranes.

Assay Procedure
Manual Procedure
1. Pipette 270 μL R1 into cuvette, to which 4 μL of sample, standard, or water (as blank) is added.
2. Incubate at 37°C for 3 minutes and blank (autozero) absorbance at 405nm.
3. Pipette 90 μL of R2 into the cuvette, mix and immediately monitor the absorbance at 405 nm for 2 minutes.
4. Calculate ΔA405/min for sample, blank, and standard by subtracting O.D. value at 60 seconds from O.D. value at 120 seconds.
ΔA405/min = (O.D. at 120sec – O.D. at 60sec)
5. Determine total bile acids concentration using the equation below:
Sample (TBA, μmole/L) = (Sample ΔA405nm/min - Blank ΔA405nm/min) x Standard
Standard ΔA405nm/min - Blank ΔA405nm/min
If sample bile acids exceed linear range (1-180 μmole/L), dilute sample with 0.9% NaCl before assay.
Test Scheme for Chemistry Analyzers

Application sheets for use of Diazyme Total Bile Acids Enzymatic Cycling Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

Calibration
A bile acids calibrator is included with the reagents and, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure. Daily calibration is required.

Quality Control
We recommend that each laboratory use bile acid controls to validate the performance of bile acid reagents. A set of normal and abnormal range bile acid controls is available from Diazyme Laboratories (Cat. # DZ042A-Con). If the results from the controls fall outside the acceptable limits, as determined by the manufacturer, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines or accreditation requirements.

Results
Bile acids concentration is expressed as μmol/L (μEq/L).

Reference Range
Serum or plasma containing 0-10 μmol/L bile acids is considered normal range. We suggest that each laboratory establish a normal range first before analyzing patient sample sets.

Limitations
- A sample with a bile acids level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.
- The linearity of the procedure is from 1 to 180 μmol/L.

Performance Characteristics
These performance characteristics were determined at Diazyme Laboratories using automated procedures on Hitachi 717.

Accuracy
The performance of this assay was compared with the performance of a similar total bile acids assay on a Hitachi 717 analyzer using serum samples.

Fifty-two (52) serum samples ranging from 0.47 – 131.25 μmol/L gave a correlation coefficient of 0.9918. Linear regression analysis gave the following equation:

\[ \text{This method} = 1.1536 \times \text{reference method} - 0.8567 \text{μmol/L} \]

A matched set of serum and lithium heparin plasma samples ranging from 0.14 – 21.18 μmol/L gave a correlation coefficient of 0.9805. Linear regression analysis gave the following equation:

\[ \text{Lithium heparin} = 0.9972 \times \text{serum} + 0.1178 \text{μmol/L} \]

Precision Studies
The intra-assay precision and inter-assay precision were evaluated in samples containing two different bile acid levels (8 μM and 23 μM). The inter-assay precision was evaluated by testing these two level specimens (low = 8 μM and high = 23 μM) in 20 runs. All tests were done using the Hitachi 717 Auto-analyzer instrument. Precision data is summarized in the table below:

<table>
<thead>
<tr>
<th>Precision Studies</th>
<th>Level 1 (8 μM)</th>
<th>Level 2 (23 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>7.93</td>
<td>23.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td>CV%</td>
<td>3.9%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Assay Precision</th>
<th>Level 1 (8 μM)</th>
<th>Level 2 (23 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>8.12</td>
<td>23.0</td>
</tr>
<tr>
<td>SD</td>
<td>0.24</td>
<td>0.61</td>
</tr>
<tr>
<td>CV%</td>
<td>2.9%</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

Linearity
Six levels of a bile acid linearity set were tested on a Hitachi 717 analyzer. The test results showed that Diazyme Total Bile Acids assay has a linear range from 1 to 180 μM with recovery averaged 98.6%.

Interference
Interference for the Diazyme Total Bile Acids Assay was evaluated on a Hitachi 717 analyzer. The following substances normally present in serum produced less than 1% deviation at the listed concentrations: Triglycerides at 750 mg/mL, Ascorbic acid at 50 mg/dL, Bilirubin at 50 mg/dL and Hemoglobin at 500 mg/dL.

References