**Total Bile Acid Assay Kit (TBA)**

**Method:** Enzymatic recycling

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB060G</td>
<td>R1: 6x60 ml, R2: 6x20 ml</td>
<td>For Hitachi 717 &amp; Shimadzu CL7200/8000</td>
</tr>
<tr>
<td>GB060G/S</td>
<td>R1: 2x60 ml, R2: 2x20 ml</td>
<td>For Hitachi 717 &amp; Shimadzu CL7200/8000</td>
</tr>
<tr>
<td>GS061G</td>
<td>R1: 6x60 ml, R2: 6x20 ml</td>
<td>For Hitachi917 &amp; Olympus AU 640/400/600</td>
</tr>
<tr>
<td>GS061G/S</td>
<td>R1: 2x60 ml, R2: 2x20 ml</td>
<td>For Hitachi917 &amp; Olympus AU 640/400/600</td>
</tr>
<tr>
<td>GH061G</td>
<td>R1: 2x48 ml, R2: 2x16 ml</td>
<td>For Hitachi902</td>
</tr>
<tr>
<td>GX061G</td>
<td>R1: 2x60 ml, R2: 2x20 ml</td>
<td>For SYNCHRON CX4-5-7-9/LX20/DXC600-800</td>
</tr>
<tr>
<td>GT061G</td>
<td>R1: 5x42 ml, R2: 2x35 ml</td>
<td>For TOSHIBA</td>
</tr>
<tr>
<td>GD061G</td>
<td>R1: 24x3.8 ml, R2: 12x2.6 ml</td>
<td>For DATE DEMENSION</td>
</tr>
</tbody>
</table>

**INTENDED USE**
For the *in vitro* quantitative determination of bile acid in serum or plasma.

**CLINICAL SIGNIFICANCE**

The assay kit is for determination of serum total bile acids (TBA). 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 stable liquid formulation that allows ease of use coupled with enhanced performance characteristics. In the presence of Thio-NAD, the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3α-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.

**SAMPLE COLLECTION AND PREPARATION**

Serum or plasma samples. Use fresh patient serum or EDTA treated plasma samples. TBA concentration is increased after meals, hence sample should be collected under fasting condition. Serum or plasma samples are stable for a week at 2-8 °C, or for 3 months at -20 °C.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Contents</th>
<th>Concentration of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 (R1)</td>
<td></td>
</tr>
<tr>
<td>Goods buffer</td>
<td></td>
</tr>
<tr>
<td>Oxidized-thio niacinamide urea</td>
<td>952.9 mg/dl</td>
</tr>
<tr>
<td>Thio-NAD</td>
<td></td>
</tr>
<tr>
<td>Reagent 2 (R2)</td>
<td></td>
</tr>
<tr>
<td>Goods buffer</td>
<td></td>
</tr>
<tr>
<td>NADH</td>
<td>6.1 g/L</td>
</tr>
<tr>
<td>3α-HSD</td>
<td>12500 U/L</td>
</tr>
<tr>
<td>Sodium azide</td>
<td></td>
</tr>
</tbody>
</table>

**STABILITY AND PREPARATION OF REAGENTS**

All reagents are ready to use. Stable up to the expiry date when stored at 2-8 °C. The reagents are stable for 1 month on board the analyser after opening and kept at 2-8°C.

**ASSAY PROCEDURE**

**Test Procedure for Analyzers (HITACHI 917)**
Assay Mode: 2 Point Rate, 21-29
Wave Length (main/sub): 405 nm/600 nm

Sample: 3 μl
R1: 225 μl, R2: 75 μl

**Graph:**

1. Mix 3 μl sample with 225 μl R1 and incubate at 37 °C for 5 minutes.
2. Add 75 μl R2 into cuvette, mix and incubate for 1 minute at 37 °C.
3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes.
4. Calculate absorbance change per minute (ΔA/min).
**CALCULATION**

\[
\text{Concentration} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{calibrator}}} \times \text{Calibrator value}
\]

**CALIBRATION**

Recommend that this assay should be calibrated using Gcell Calibrator (Cat.No. GC-TBA).

**QUALITY CONTROL**

Randox Assayed Multi- sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

**NORMAL VALUE**

Serum or plasma: 0.5-10 μmol/L.

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

**LINEARITY**

The method is linear up to 200 μmol/L. Samples above this concentration should be diluted with 0.9% NaCl and re assay. Multiply the result by dilution factor.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**INTERFERENCE**

The following analytes were tested up to the levels indicated and found not to interfere:

- Hemoglobin: 500 mg/dl
- TG: 2000 mg/dl
- Direct bilirubin: 50 mg/dl
- Ascorbic Acid (VC): 10 mg/dl

**PRECISION**

The CV of the test should be less than 5%.

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (μmol/L)</td>
<td>25.8</td>
<td>40.94</td>
</tr>
<tr>
<td>SD</td>
<td>0.33</td>
<td>0.39</td>
</tr>
<tr>
<td>CV</td>
<td>1.30%</td>
<td>0.96%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (μmol/L)</td>
<td>25.97</td>
<td>40.08</td>
</tr>
<tr>
<td>SD</td>
<td>0.26</td>
<td>0.58</td>
</tr>
<tr>
<td>CV</td>
<td>1.02%</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

**SENSITIVITY**

The minimum detectable concentration of Total Bile Acids with an acceptable level of precision was determined as 2.13 μmol/L.

**CORRELATION**

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

\[
Y=1.007X-0.05,
\]

and a correlation coefficient of r=0.997; 80 patient samples were analyzed.

**SAFETY PRECAUTIONS AND WARNINGS**

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
2. The reagents contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.
REFERENCES
2. Sverre Skrede, Helge Erik Solberg, Jan Petter Biomhoff, and Egli Gjone Bile Acids Measured in Serum during Fasting as a Test for Liver Disease CLIN.CHEM.24/7, 1095-1099 (1978)
7. Michael J. Croweli and Ian A. Macdonald1 Enzymic Determination of 3a-, 7a-, and 12a-Hydroxyl (loops of Fecal Bile Salts. CLIN. CHEM. 26/9, 1298-1300 (1980)1298

INDEX OF SYMBOLS
- Manufacture
- Catalogue Number
- Lot number
- Date of manufacture
- Use by (Expiration date)
- For In-Vitro Diagnostic use only
- Stored at 2-8°C
- Attention: See instruction for use
- Authorized Representative in the European Company