Quantitative determination of Total Bile Acids

**IVD**

Store at 2-8°C

**INTENDED USE**

For the quantitative in vitro determination of Total Bile Acids in serum and plasma.

**PRINCIPLE OF THE METHOD**

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 excesses 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.

Thio-NAD → Thio-NADH

Bile acids → Oxidised bile acids

3-α HSD → NAD

**CLINICAL SIGNIFICANCE**

Fasting serum bile acids can be used in the diagnosis and prognosis of liver disease. Levels rise in many liver diseases, for example hepatitis and liver sclerosis. Abnormal levels in fasting patients or immediately after a meal can be used to detect liver disease and damage, impaired liver function, intestinal dysfunction and perhaps a gall bladder blockage. Bile acid measurement may detect some forms of liver disease earlier than standard liver tests because bile acids levels correspond to liver function, rather than liver damage. In veterinary medicine, bile acid measurement is considered to be a superior indicator of liver disease.

**REAGENTS**

<table>
<thead>
<tr>
<th>R1</th>
<th>Goods buffer, pH 4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thio-NAD</td>
</tr>
<tr>
<td></td>
<td>Triton-100</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>R2</th>
<th>Goods buffer, pH 9.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAD</td>
</tr>
<tr>
<td></td>
<td>3-α HSD</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
</tr>
</tbody>
</table>

**PRECAUTIONS**

R1 and R2 contain 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. See MSDS for disposal of the product.

**CALIBRATION**

Total Bile Acids Calibrator ref. 1002290 is recommended for calibration. Recalibration is recommended daily.

**PREPARATION**

Reagents are ready for use.

**STORAGE AND STABILITY**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contamination prevented during their use. Do not use reagents over the expiration date. Stability: Once opened R1 and R2 are stable for 28 days at 2-8°C.

**ADDITIONAL EQUIPMENT**

- Spectrophotometer or colorimeter measuring at 405 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**SAMPLES**

- Serum, EDTA / Lithium heparin plasma. Serum or plasma samples are stable for 1 week at 2-8°C, or at 3 months at -20°C.

**PROCEDURE**

1. **Assay conditions:**
   - Wavelength: 405 nm
   - Cuvette: 1 cm light path
   - Constant temperature: 37°C / 15-25°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

   | R1 Standard Sample |
   |-------------------|------------------|
   | 750 µL            | 750 µL           |
   |
   | R2 Standard Sample |
   | 250 µL            | 250 µL           |
   |
   | Standard          | Sample           |
   | 10 µL             | 10 µL            |

4. Mix and read the absorbance after 60 s (A1) and 120 s (A2).

5. Calculate: \( \Delta A = A_2 - A_1 \).

**CALCULATIONS**

\( \frac{\Delta A}{\text{Calibrator conc}} = \text{µmol/L bile acid in the sample} \)

**QUALITY CONTROL**

Control Sera are recommended to monitor the performance of assay procedures: TBA / CO2. Control Ref. 1002292. If control values are found outside the defined range, check the instrument, reagent and calibration for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**REFERENCE VALUES**

Human Serum (fasting): 0 - 10 µmol/L

These values are for orientation purpose; each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

**Linearity:** The method is linear up to a concentration of 150 µmol/L. In the event of a rerun, the linearity is extended to 225 µmol/L.

**Sensitivity:** The minimum detectable level that can be distinguished from zero has been determined as 1.47 µmol/L.

**Precision:**

<table>
<thead>
<tr>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µmol/L)</td>
<td>8.96</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.17</td>
</tr>
</tbody>
</table>

The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**

The following analytes were tested up to the levels indicated and were found not to interfere: Haemoglobin (250 mg/dL), Triglycerides (1000 mg/dL), Intralipid (800 mg/dL), and Bilirubin (85 mg/dL).

**NOTES**

1. The reagent should not be used if exposed to temperatures above 25°C for greater than 8 hours, as the accuracy of the assay will be affected.

2. **SPINREACT** has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**BIBLIOGRAPHY**


**PACKAGING**

Ref: 1001030

Cont.

R1: 1 x 50 mL, R2: 1 x 18 mL