Total bile acids (TBA) diagnostic reagent is used for quantitative in vitro determination of total bile acids in serum or plasma on photometric systems.

**Background**

**Liver:** Total bile acids are metabolized in the liver and hence serve as a marker for normal liver function. Total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer, liver cirrhosis, Cholestasis, Congenital and acquired vascular shunts.

**Pregnancy:** Obstetric cholestasis is the most common liver disease seen during pregnancy and the test is a diagnostic tool for investigating suspected cases. The main symptom is itching, but tiredness, nausea and jaundice may also be reported. In addition to the discomfort experienced by the mother, high levels of bile acids also increased risk to the baby and may lead to premature birth or greater risk of passing meconium in the womb.

**Veterinary:** In veterinary medicine the bile acids test is typically used to assess liver function in mammals such as cats, dogs, horses, pigs, cows and sheep. The test is also used in the investigation of avian liver disease, where elevated liver enzymes do not always correlate with liver disease. In the most species a random sample is assessed but a dual sampling protocol is typically used with cats and dogs to investigate bile reabsorption. For the latter a fasting blood sample is taken and then a second, post prandial sample taken a few hours after being fed for comparison. Total bile acids are typically low in a fasted state, whilst level will rise immediately after a meal. When the liver has normal mass and blood flow the bile acid levels will rapidly fall back to near fasting level as the liver removes them from the blood and returns them to the gall bladder.

**Method**

Enzymatic recycling method

**Assay Principle**

In the presence of Thio-NAD, the enzyme 3-á hydroxysteroid dehydrogenase (3-á HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-á 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 405nm.

**Reagents**

<table>
<thead>
<tr>
<th>Reagent 1(R1)</th>
<th>Reagent 2(R2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (pH 7.0)</td>
<td>Buffer (pH 7.0)</td>
</tr>
<tr>
<td>Thio-NAD</td>
<td>Thio-NAD</td>
</tr>
<tr>
<td>65 mmol/L</td>
<td>65 mmol/L</td>
</tr>
<tr>
<td>1 g/L</td>
<td>6 g/L</td>
</tr>
</tbody>
</table>

**Precautions and Warnings**

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Reagent Preparation, Storage and Stability**

Spectrum TBA reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles at 2 – 8 °C.

**Deterioration**

Failure to recover control values within the assigned range may be an indication of reagent deterioration.

**Specimen Collection and Preservation**

The only acceptable anticoagulat is EDTA. Use preferably fresh serum.

**Stability:** 1 week at 4 – 8 °C

2 months at -20 °C

**System Parameters**

- **Wavelength:** 405 nm
- **Optical path:** 1 cm
- **Assay type:** Fixed rate
- **Direction:** Increase
- **Temperature:** 37 °C
- **Incubation time:** 5 minutes at 20–25 °C
- **Zero adjustment:** Reagent Blank
- **Sensitivity:** 0.5 µmol/L
- **Linearity:** 150 µmol/L

**Procedure**

<table>
<thead>
<tr>
<th>Blank</th>
<th>Calibrator</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent1(R1)</td>
<td>300 µl</td>
<td>300 µl</td>
</tr>
<tr>
<td>Calibrator</td>
<td>-----</td>
<td>5 µl</td>
</tr>
<tr>
<td>Specimen</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 minutes at 37°C then add:

| Reagent2(R2) | 100 µl | 100 µl | 100 µl |

Mix, and after 1 minute read the absorbance A1 of the Calibrator or specimen. After 3 minutes later, read absorbance A2 of calibrator or specimen.

**Calculation**

A2 – A1 = Aspecimen - Ablank

TBA(µmol/L) = \frac{Aspecimen - Ablank}{Acalibrator - Ablank} \times \text{conc. of calibrator}

**Quality Control**

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

**Performance Characteristics**

**Precision**
Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µmol/L)</td>
<td>45.54</td>
<td>171.62</td>
</tr>
<tr>
<td>SD</td>
<td>0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>CV%</td>
<td>0.76</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µmol/L)</td>
<td>85.16</td>
<td>228.18</td>
</tr>
<tr>
<td>SD</td>
<td>3.25</td>
<td>6.69</td>
</tr>
<tr>
<td>CV%</td>
<td>3.82</td>
<td>2.93</td>
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</table>

Calibration

The assay requires the use of a total bile acids calibrator. Recalibration is recommended at anytime if one of the following events occurs:
* The lot number of reagents changes.
* Preventative maintenance is performed or a critical component is replaced.
* Control values have shifted or are out of range and a new vial of control does not rectify the problem.

Methods Comparison

A comparison between Spectrum Diagnostics TBA reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.97 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay 0.5 µmol/L.

Linearity

The reaction is linear up to an albumin concentration of 180 µmol/L. Specimens showing higher concentration should be diluted 1+1 with physiological saline and repeat the assay (result × 2).

Interfering Substances

Serum, plasma

Haemolysis

A haemoglobin level of 150 mg/dL results in 13% positive bias.

Icterus

No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia

No significant interference up to an intralipid level of 2%.

Expected Values

0.0 - 10.4 µmol/L

It is recommended that each laboratory should establish its own reference interval.

Analytical Range

0.5 – 180 µmol/L.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

References

1. Abbassi-Ghanavati M, Greer LG, Cunningham FG.
2. Wallach, J. Eighth ed. Lippincott Williams & Wilkins

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
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<tbody>
<tr>
<td>305 001</td>
<td>100 test</td>
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