Enzymatic colorimetric determination of total bile acids in serum

**REAGENT 1a:** 5 x 10 mL  -  **REAGENT 1b:** (lyophilized) 5 x 10 mL
**REAGENT 2:** 1 x 13 mL  -  **STANDARD:** 1 x 5 mL

**PRECAUTIONS IN USE**
In addition to the possible risks regarding the reactive components, product may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 1999/45/EC directives and modifications and amendments regarding classification, labelling and packaging of dangerous preparations (reagents) have been made accordingly. However, it is recommended that this product be handled carefully, that ingestion and contact with eyes, skin and mucous membranes be avoided and that laboratory reagents are used according to good laboratory practice.

**SUMMARY**
Total bile acids are metabolized in the liver and hence serve as a marker for liver function. Serum total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer.

**PRINCIPLE**
In the presence of NAD, the enzyme 3-alpha hydroxysteroid dehydrogenase (3-alpha HSD) converts bile acids to 3-keto steroids and NADH. The NADH formed reacts with nitrotetrazolium blue (NBT) to form a formazan dye in the presence of diaphorase enzyme. The colour intensity of the formazan dye is proportional to the bile acids concentration in the sample.

**REAGENTS**
Reagents, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.

- **REAGENT INFORMATION:**
  - the STANDARD value is verified using an internal standard, obtained by weighing purified material.
  - REAGENT 1a must be clear; do not use if turbid.
  - the Solution R1 has a typical yellow coloration.

Preparation of reagent solutions
Solution R1: reconstitute the contents of one vial of REAGENT 1b with the contents of one vial of REAGENT 1a; leave to stand for about 10 minutes and gently mix. Stability: 7 days at 2-8 °C or 4 weeks at -20 °C, if contamination is avoided and vials are recapped immediately after use.

**REAGENT 2:** ready to use. The reagent in an unopened vial is stable up to the expiry date indicated on the package. Stability: 90 days at 2-8 °C after opening, if contamination is avoided and the vial is recapped immediately after use.

**STANDARD:** ready to use. The reagent in an unopened vial is stable up to the expiry date indicated on the package. Stability: 120 days at 2-8 °C after opening, if contamination is avoided and the vial is recapped immediately after use.

**QUALITY CONTROL**
The use of following control materials at different levels of analyte is recommended to verify test accuracy:

- **Bile Acids Controls:**
  - Lyophilized controls. For use, follow the instructions contained in the kit.

**SAMPLE**
Fresh serum. Avoid hemolysed or highly lipaemic samples. Collect samples in accordance with the NCCLS procedure reported in the bibliography (1). Stability of the sample: 5 days at 2-8 °C.

**ANALYTICAL PROCEDURE**
Wavelength: 546 (540-570) nm
Pathlength: 1 cm
Temperature: 37 °C
Sample/Solution R1/REAGENT 2: 1/2/0.5
Reaction: fixed-time (increase)
Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

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**Put into cuvette**  
Sample Blank | Sample | Standard Blank | Standard
---|---|---|---
Sample | 0.2 mL | 0.2 mL | - | -
STANDARD | - | 0.2 mL | 0.2 mL | 0.2 mL
Solution R1 | 0.4 mL | 0.4 mL | 0.4 mL | 0.4 mL

Mix carefully and incubate for 5 minutes at working temperature. Add:

**REAGENT 2** - | 0.1 mL | - | 0.1 mL | -
Distilled Water | 0.1 mL | - | 0.1 mL | -

Mix carefully and incubate for exactly 5 minutes at working temperature. Immediately read the absorbance of Sample (AS) and Standard (AST) against Sample Blank (ABS) and Standard Blank (ABST).

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**CALCULATION**

\[(AS - ABS) / (AST - ABST) \times [STD]^* = \mu\text{mol bile acids / L sample}\]

* = bile acids concentration (µmol/L) indicated on the standard vial label

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**REFERENCE VALUES**

Serum (fasting): 2 - 10 µmol/L

It is recommended that each laboratory establish its own expected range.

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**PERFORMANCES**

(determined on Hitachi automatic analyzer)

**Interferences**: the test is not affected by the presence of bilirubin up to 20 mg/dL, hemoglobin up to 1 g/dL and triglycerides up to 250 mg/dL.

**Measuring range**: 1.0 - 200 µmol/L. Samples with concentration higher than 200 µmol/L must be diluted 1:10 with normal saline and result multiplied by 10.

**Intra-Assay Precision**: it was determined on 20 replicates of each control (2 levels - L1/L2). Results were as follows: L1: average 6.59 µmol/L, SD 0.14, CV% 2.20 / L2: average 73.58 µmol/L, SD 0.67, CV% 0.91.

**Inter-Assay Precision**: it was determined for 10 days on 2 replicates of each control (2 levels - L1/L2). Results were as follows:

<table>
<thead>
<tr>
<th>Mean</th>
<th>Within run</th>
<th>Run to run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/L</td>
<td>SD</td>
<td>CV%</td>
<td>SD</td>
</tr>
<tr>
<td>L1</td>
<td>6.63</td>
<td>0.17</td>
<td>2.56</td>
</tr>
<tr>
<td>L2</td>
<td>74.40</td>
<td>1.35</td>
<td>1.82</td>
</tr>
</tbody>
</table>

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**Sensitivity**: 1.0 µmol/L. Sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value + 3 SD".

**Accuracy**: this test (y) was compared with a commercially available method (x). Results were as follows:

\[N = 42, \ r = 0.985, \ y = 0.98x + 0.89\]

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**WASTE MANAGEMENT**

Reagents must be disposed of in accordance with local regulations.

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**BIBLIOGRAPHY**