Principle:
Lipids from blood serum are extracted by the mixture ethanol/diethyleter (3:1). To 1 ml of serum 9 ml of mixture ethanol/diethyleter is added and mixed. After centrifugation clean extract we use for lipid determination.

We determine unesterified fatty acids in three situations:
  a) after carbohydrate meal
  b) fasting
  c) in diabetics

Principle: Unesterified fatty acids are determined by neutralization titration with solution of NaOH and using indicator bromothymol blue (in acidic environment is yellow coloured, in alkaline environment is blue).

Procedure:

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipidic extract 1</td>
<td>0,5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipidic extract 2</td>
<td></td>
<td>0,5</td>
<td></td>
</tr>
<tr>
<td>Lipidic extract 3</td>
<td></td>
<td></td>
<td>0,5</td>
</tr>
<tr>
<td>Bromothymol. blue</td>
<td>0,1</td>
<td>0,1</td>
<td>0,1</td>
</tr>
</tbody>
</table>

Titration with solution of NaOH
- NaOH we add carefully by drops, with continuous mixing
- we titrate until change to blue colour which stays minimum 5 seconds
- every titration we repeat two times, for calculation we use average from two titrations
- amount of used NaOH – 1 drop = 50 μl

From calibration graph according to used NaOH we read amount of unesterified fatty acids in the sample and calculate concentration of fatty acids in mmol/l.

Physiological values: 0.3 – 1.2 mmol/l

Conclusion: