INTRODUCTION
The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology. Lipids react with sulfuric acid to form carbonium ions, which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically.

In blood, at least 95% of the lipids exist in combination with protein. These lipoproteins can be quantitated by disrupting this complex. This test is used as a screening method for hyperlipidemia. The sulfo-phospho-vanillin (SPV) method for the colorimetric determination of the serum total lipids was described by Charbrol et al. and modified by several investigators by omitting phosphoric acid, shortening reaction time, and increasing reagent stability. Our reagent is based on all of these modifications.

PRINCIPLE
Unsaturated lipids react with sulphuric acid to form carbonium ions. In a second step the carbonium ions react with phosphovainilline to give a pink colour. The intensity of the color formed is proportional to the total lipids concentration in the sample.

MATERIALS REAGENTS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Phosphovainilline</th>
<th>235 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphoric acid</td>
<td>2 mmol/L</td>
</tr>
<tr>
<td>Standard</td>
<td>Total Lipids aqueous primary standard</td>
<td>750 mg/dL</td>
</tr>
<tr>
<td>Sulfuric Acid</td>
<td>Needed reagent but not provided.</td>
<td></td>
</tr>
</tbody>
</table>

MATERIALS REQUIRED BUT NOT PROVIDED
- Concentrated sulfuric acid.
- Pipettes.
- Test vials or cuvettes.
- Timer.
- 100°C heating bath.
- Control serum.
- Spectrophotometer.
- Sulphuric Acid (H₂SO₄).

PREPARATION
Reagent and standard are ready to use.

PRECAUTIONS
- Exercise the normal precautions required for handling of all laboratory reagents.
- Pipetting by mouth is not recommended for any laboratory reagent. Concentrated sulfuric acid causes severe burns and eye damage.

STORAGE AND STABILITY
- All reagents are stable until the expiration date indicated on label.
- Total lipid Reagent Store at 2-8°C.
- Protected from light.
- Stability of the sample: Total lipids are stable 24 h at room temperature (15-25°C) or 3 days at 2-8°C.
- Signs of reagent deterioration:
  o Presence of particles and turbidity.
  o Blank absorbance (A) at 520 nm ≥ 0.32.

SPECIMEN COLLECTION
- Serum or Plasma.

PROCEDURES
1. Assay conditions:
   Wavelength: 520 nm (490-550).
   Cuvette: 1 cm light path.
   Temperature: 37°C.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a glass tube:

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂SO₄ (mL)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Standard (µL)</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Sample (µL)</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Mix and incubate for 10 minutes at 100°C in a boiling water bath.
   **Note:**
   - H₂SO₄ with Standard give Standard Acid digest (Solution A).
   - H₂SO₄ with Sample give Sample Acid digest (Solution B).
5. Cool in iced water and transfer from (Table 1) into a cuvette in (Table 2):

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (mL)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample Acid digest (µL) (Solution B)</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Standard Acid digest (µL) (Solution A)</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>

6. Mix and incubate for exactly 15 minutes at 37°C.
7. Read the absorbance (A) of the samples and standard, against the Blank. The color is stable for at least 1 hour.
CALCULATIONS:

\[(A) \text{ Sample} - (A) \text{ Blank}) \times 750 \text{ (Conc. of STD)} = \]
\[(A) \text{ Standard} - (A) \text{ Blank} \]

Total Lipid in sample (mg/dl)

REFERENCE VALUES
Serum or Plasma:
450-800 mg /dL

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

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 7.7 mg/dL to linearity limit of 1500 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/L)</td>
<td>555</td>
<td>553</td>
</tr>
<tr>
<td>SD</td>
<td>15.9</td>
<td>7.62</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.87</td>
<td>5.87</td>
</tr>
</tbody>
</table>

Sensitivity:

1 mg/dL = 0,00066 A.

Accuracy:

Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following: Correlation coefficient (r): 0.984. Regression equation: y=0.967x + 24, 08.

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

NOTES

- TOTAL LIPIDS CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.

REFERENCES