TRIGLYCERIDES GPO Method

Reagent for quantitative determination of triglycerides in human serum or plasma

| REF 80019 | R1 2 x 50 mL | R2 2 x 50 mL | R3 1 x 5 mL |
| REF 87319 | R1 10 x 100 mL | R2 10 x 100 mL | R3 1 x 5 mL |

TECHNICAL SUPPORT AND ORDERS
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CLINICAL SIGNIFICANCE (1)
The measurement of the concentration in blood triglycerides is important for the diagnosis and the follow-up of hyperlipidemia. Its increase can be of genetic origin or secondary to other metabolic disorders such as: diabetes mellitus, hyper and hypothyroidisms, hepatic diseases, acute and chronic pancreatitis, nephrosis. A rise in triglycerides also represents an atherogenic risk factor. It is responsible for the opalescence, or even the cloudiness of the serum. Corticoids and oestrogen/progestin treatments can also aggravate hypertriglyceridemia.

PRINCIPLE (4) (5)
Fossati and Prencipe method associated with Trinder reaction. Reaction scheme is as follows:

\[
\text{Lipase} \rightarrow \text{Glycerol} + \text{free fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol 3 Phosphate} + \text{ADP}
\]

\[
\text{Glycerol 3 Phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{DihydroxyacetonePhosphate} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-Chlorophenol} + \text{PAP} \xrightarrow{\text{POD}} \text{Quinoneimine (pink)} + \text{H}_2\text{O}
\]

The absorbance of the coloured complex (quinoneimine), proportional to the amount of triglycerides in the specimen, is measured at 500 nm.

SAFETY CAUTIONS
BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION
Vial R2: Use a non-sharp instrument to remove aluminium cap.
Add promptly the contents of vial R2 (Enzymes), into vial R1 (Buffer).
Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

STABILITY AND STORAGE
Store away from light, well cap in the original vial at 2-8°C.
- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 1 year when free from contamination.
- Discard reagent if cloudy or if absorbance at 500 nm > 0.200.
- Don’t use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)
Serum or plasma (Heparin or EDTA) fasting > 12 hours.
Separate from cells within 2 hours.
Do not use oxalate, fluoride or citrate.
Triglycerides are stable in specimen for:
- 5-7 days at 2-8°C.
- 3 months at ~20°C.
- many years at ~70°C.
Avoid repeated freezing and thawing.

<table>
<thead>
<tr>
<th>Vial R1</th>
<th>BUFFER</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIPES</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>9.8 mmol/L</td>
</tr>
<tr>
<td>Chloro-4-phenol</td>
<td>3.5 mmol/L</td>
</tr>
<tr>
<td>Preservative</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R2</th>
<th>ENZYMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase</td>
<td>1000 IU/L</td>
</tr>
<tr>
<td>Peroxydase (POD)</td>
<td>1700 IU/L</td>
</tr>
<tr>
<td>Glycerol 3 phosphate oxidase (GPO)</td>
<td>3000 IU/L</td>
</tr>
<tr>
<td>Glycerol Kinase (GK)</td>
<td>660 IU/L</td>
</tr>
<tr>
<td>4 - Amino – antipyrine (PAP)</td>
<td>0.5 mmol/L</td>
</tr>
<tr>
<td>Adenosine triphosphate Na (ATP)</td>
<td>1.3 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R3</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>2.28 mmol/L</td>
</tr>
<tr>
<td>Equivalent to triolein or triglycerides 200 mg/dL (2.28 mmol/L)</td>
<td></td>
</tr>
</tbody>
</table>
**INTERFERENCES (1) (2) (3)**

Ascorbic acid: No significant interference up to 2.5 mg/dL. Above, under-estimation.

Hemoglobin: No significant interference up to 1.93 g/dL (300 µmol/L).

Bilirubin: No significant interference up to 8 mg/dL (137 µmol/L) of bilirubin. Above, under-estimation.

Free glycerol: Overestimation approximately 10 mg/dL (0.11 mmol/L), generated by endogenous glycerol.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

**CALIBRATION (7)**

- Standard (vial R3) provided in the kit or BIOLABO Multicalibrator REF 95015 traceable to SRM 909b.
- Or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent. It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of ranges, even after using a new vial of fresh serum.

**QUALITY CONTROL**

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
1. At least once a run.
2. At least once within 24 hours.
3. When changing vial of reagent.
4. After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

**EXPECTED VALUES (6)**

<table>
<thead>
<tr>
<th>Triglycerides</th>
<th>mg/dL</th>
<th>[ mmol/L ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference range</td>
<td>35-160</td>
<td>[ 0.40-1.82 ]</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population it serves.

**PERFORMANCE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Within Run</th>
<th>Normal level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 30</td>
<td>Mean mg/dL</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>S.D. mg/dL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between Run</th>
<th>Normal level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 33</td>
<td>Mean mg/dL</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>S.D. mg/dL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Detection limit: approximately 10 mg/dL

Sensitivity for 100 mg/dL: approximately 0.125 Abs. at 500 nm.

Comparison with a commercially available reagent:

\[ y = 1.0182 x - 3.02 \quad r = 0.9958 \]

**LINEARITY**

The reaction is linear up to at least 700 mg/dL (7.9 mmol/L). Above, dilute the specimen with saline solution and re-assay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

**MANUAL PROCEDURE**

Let stand reagent and specimens at room temperature.

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Demineralised water</td>
<td>10 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>10 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>10 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix. Let stand for 5 minutes at 37°C or 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against reagent blank. Reaction is stable for 1 hour.

**CALCULATION**

Calculate the result as follows:

\[ \text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration} \]

**REFERENCES**

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-573 to 3-589
(7) SRM: Standard Reference Material