Estimation of Lactic Acid

Dr. Mahesh H B, Yuvaraja’s College, University of Mysore, Mysuru.

Aim: To estimate the amount of lactic acid.

Principle: The lactic acid is oxidized to acetaldehyde by sulphuric acid in the presence of copper sulphate, a purple colour is developed with \( p \) – hydroxydiphenyl. This colour can be measured at 650 nm.

Requirements:
1. 20 % Copper sulphate solution.
2. 4 % Copper sulphate solution.
3. Calcium hydroxide.
4. Sulphuric acid.
5. Parahydroxydiphenyl reagent: Dissolve 1.5 gm of PHDP in 1 ml of 5% NaOH by warming and stirring, and make up to 10 ml with water.
6. Standard lactic acid solution: Dissolve 34 mg of lithium lactate in 10 ml of distilled water in 100 ml volumetric flask. Add 0.1 ml of concentrated sulphuric acid and make up to the mark. This solution contains 0.2 mg per ml or 1mg in 5 ml.

For preparing the working standard solution, dilute this stock solution in the ratio of 1:10. This working solution contains 20µg/ml

Preparation of protein free filtrate: To 1 ml blood sample, add 8 ml distilled water, 0.5 ml of 2/3 N sulfuric acid and 0.5 ml of 10% sodium tungstate solution in a stoppered centrifuge tube and mix the contents. Then centrifuge at 3000 rpm for 10 min and collect the supernatant as sample.

Procedure:
1. To 1 ml of protein free filtrate, add 1 ml of 20 % copper sulphate solution and make up the solution to 10 ml. Then add 1 gm of powdered calcium hydroxide, shake well until the contents dispersed uniformly. Keep the test tubes at room temperature for 1 hr with interim shaking and centrifuge the contents.
2. Take 1 ml of supernatant in a clean test tube and add 0.25 ml of 4% copper sulphate solution followed by 6 ml of concentrated sulphuric acid. Mix the contents well by lateral shaking. Then keep the tube in a boiling water bath for 6.5 min. Then cool the contents, add 0.1 ml of PHDP and mix well. Place the test tubes at room temperature for 30 min.
3. Now keep the tubes in a boiling for exactly 90 sec, cool to room temperature and read at 650 nm against the blank.
4. For standard curve take 0, 1, 2, 3, 4, 5 ml of working standard solution and treat as above.
5. Plot the standard curve by taking concentration of lactic acid along X-axis and absorbance at 650 nm along Y-axis.
6. Then from this standard curve calculate the concentration of lactic acid in the given sample.

Observations and Calculations

<table>
<thead>
<tr>
<th>Volume of standard Lactic acid (20µg/ml)</th>
<th>Concentration of Lactic acid (µg)</th>
<th>Volume of 20% CuSO₄ solution (ml)</th>
<th>Amoun of CaOH (gm)</th>
<th>Volume of supernatant (ml)</th>
<th>Volume of 4% CuSO₄ solution (ml)</th>
<th>Volume of H₂SO₄ (ml)</th>
<th>PHDP (ml)</th>
<th>Incubate for 6.5 min in a boiling water bath</th>
<th>PHDP + Incubate at room temp for 30 min</th>
<th>PHDP + Incubate at room temp for 90 sec</th>
<th>Incubate at room temp for 30 min followed by in boiling water bath for 90 seconds</th>
<th>A₆₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.25</td>
<td>6.0</td>
<td>0.1</td>
<td>0.1</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td>1.0 Unknown / PFF</td>
<td>To be estimated</td>
<td>1.0</td>
<td>1.0</td>
<td>0.25</td>
<td>6.0</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Result: The given unknown sample contains ----µg lactic acid / ml sample.

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