Lactate
Colorimetric LOD-PAP-Test
(Monoreagent)

METHOD/ PRINCIPLE
Enzymatic color test, Trinder type, endpoint.

Lactate Oxidase 1.1.3.2 (LOD) splits lactate into pyruvate and hydrogen peroxide which reacts with 4-aminoantipyrin and tribromo-3-hydroxybenzoic acid in the presence of peroxidase to form a red coloured quinoneimine. The increase of the absorbance at 546nm is proportional to the lactate concentration.

REAGENTS
Components (concentrations in the test)
Reagent:
Tris-Buffer (pH 7,5) 100 mmol/l
Laktat-Oxidase (LOD) > 0.3 kU/l
Peroxidase (POD) > 1.0 kU/l
4-Aminoantipyrin 0.3 mmol/l
TBHB* > 1.2 mmol/l
Sodium Azide 0.8 g/l

Standard: 30.0 mg/dl (3.32 mmol/l)

* Tribromo-3-hydroxybenzoic Acid

Preparation and Stability:
The reagent R1 is ready to use.
When still sealed it is stable up to the indicated expiry date if stored at 2° to 8°C.
When the reagent is opened the stability is still at least 6 weeks at 2° to 8°C, if contamination is avoided.

Precautions:
- For in vitro diagnostic use only.
- These reagents contain 0.8 g/L sodium azide.
  Do not swallow and avoid contact with skin and/or mucous membranes
- Avoid direct exposure to light.

SAMPLE MATERIAL
Whole Blood*, EDTA - or fluoridized plasma**, Liquor

Stability:
Plasma (with fluoride**):
8 hours at 2 – 8°C
Whole blood (deproteinized*), Liquor:
7 days at 2 – 8°C

* Deproteinize with Perchloric Acid / Perchlorate immediately after winning of blood
** Use Na- or K-Fluoride-tubes

WASTE
Handle according to the local legal regulations

ASSAY PROCEDURE:

Wavelength: 546nm Hg (495-550nm)
Light path: 1cm
Temperature: 25° or 37°C
Measurement: against reagent blank (RBL)

<table>
<thead>
<tr>
<th>Sample (S)</th>
<th>Standard (STD)</th>
<th>Reagent R1</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µL</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 min at 25°C or 37°C.
In between 30 min measure the absorbance of the sample AS and the standard A_STD against the reagent blank RBL.

CALCULATION (using standard)

Lactate (mg/dl) = \( \frac{A_S}{A_{STD}} \times 30 \)

Conversion Factor
Lactate [mg/dl] x 0,11 = Lactate [mmol/l]

CALIBRATION AND QUALITY CONTROL
For internal quality control we recommend Greiner’s control sera Unitrol-I and Unitrol-II with Lactate-PAP values, and for calibration of automated analyzers our Multicallibrator

NOTES
1. Applications for most Automated Analyzers are available on request
2. A working procedure “With Deproteinization” is available on request
PERFORMANCE DATA

Analytical Range
The test is linear up to Lactate concentrations of 200 mg/dl (22,1 mmol/l). Exceeding this range the samples should be diluted with 9% NaCl solution 1 + 1. The result has to be multiplied by 2.

Detection Limit / Sensitivity
The detection limit is 0,11 mg/dl (0,012 mmol/l).

Precision
Within-run reproducibility

<table>
<thead>
<tr>
<th></th>
<th>Mean (mg/dl)</th>
<th>SD (mg/dl)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>15,57</td>
<td>0,145</td>
<td>0,93</td>
</tr>
<tr>
<td>Control 2</td>
<td>29,08</td>
<td>0,19</td>
<td>0,65</td>
</tr>
<tr>
<td>Patient</td>
<td>17,29</td>
<td>0,11</td>
<td>0,66</td>
</tr>
</tbody>
</table>

Between-run reproducibility

<table>
<thead>
<tr>
<th></th>
<th>Mean (mg/dl)</th>
<th>SD (mg/dl)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>15,48</td>
<td>0,11</td>
<td>0,69</td>
</tr>
<tr>
<td>Control 2</td>
<td>29,19</td>
<td>0,34</td>
<td>1,16</td>
</tr>
<tr>
<td>Patient</td>
<td>17,36</td>
<td>0,12</td>
<td>0,67</td>
</tr>
</tbody>
</table>

Correlation
A comparative study has been performed between the Greiner method and another commercial reagent on 30 human samples. The parameters of linear regression are as follows:

\[ y = 1,000 \times \ldots \quad r = 0,999 \]

Interferences
No Interference with
- Ascorbic acid up to 20 mg/dl
- Bilirubine up to 40 mg/dl
- Hemoglobine up to 1000 mg/dl
- Lipemia up to 2000 mg/dl Triglycerides.

REFERENCE VALUES

<table>
<thead>
<tr>
<th></th>
<th>[mg/dl]</th>
<th>[mmol/l]</th>
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</thead>
<tbody>
<tr>
<td>Newborns (blood*)</td>
<td>&lt; 25</td>
<td>&lt; 2.8</td>
</tr>
<tr>
<td>Adults and Children:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial whole blood *</td>
<td>&lt; 13</td>
<td>&lt; 1.5</td>
</tr>
<tr>
<td>(Arterial) Plasma</td>
<td>&lt; 16</td>
<td>&lt; 1.8</td>
</tr>
<tr>
<td>Venous whole blood *</td>
<td>&lt; 20</td>
<td>&lt; 2.2</td>
</tr>
<tr>
<td>(Venous) Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquor</td>
<td>11 - 19</td>
<td>1.2 – 2.1</td>
</tr>
</tbody>
</table>

* after deproteinization – see „Notes”

BIBLIOGRAPHY
6. Barham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst 1972; 9

SYMBOLS USED

[IVD] For in vitro diagnostic medical use

[LOT] Batch Code

[ ] Use by

[ ] Temperature limitation