Reagent kit for determination of chloride ion concentration in serum, urine and cerebrospinal fluid.

A colorimetric endpoint method based on the reaction with mercuric thiocyanate.

Chloride ion represents that anion of the salt and water household which is present in the highest concentrations in the body. It is primarily found as NaCl in the extracellular compartment and as HCl in the gastrointestinal tract. Low chloride concentrations are associated with severe vomiting, diarrhoea, colitis ulceraosa, diabetic acidosis, Addison's disease. Decreased chloride concentrations are found also in cases when drugs that need chloride ions for their absorption are taken for prolonged time. Increased levels are observed in cases of dehydration, congestive heart failure, Cushing's syndrome, hyperventilation, anaemia, nephritis and renal obstruction.

**Principle**

Chloride ion in acidic environment in presence of ferric nitrate forms a colored complex with mercuric thiocyanate. Intensity of the developed colour is proportional to the chloride ion concentration in the sample.

\[ 2\text{Cl} + \text{Hg(SCN)}_2 \rightarrow \text{HgCl}_2 + 2\text{SCN}^- \]

\[ \text{SCN}^- + \text{Fe}^{3+} \rightarrow \text{Fe(SCN)}^2+ \]

**Reference values**

- **Serum**: 98-107 mmol/l (348-380 mg/dl)
- **Cerebrospinal fluid**: 119-131 mmol/l (421-465 mg/dl)
- **Urine**: 110-250 mmol/24 h (389-887 mg/24 h)

It is recommended that each laboratory should assign its own normal range.

**Samples**

Serum, urine, cerebrospinal fluid.

**PROCEDURE**

Reagent is ready for use. If the absorbance of working reagent is higher than 0.1 at 492 nm the reagent can not be used.

**Assay conditions**

- **Wavelength**: 500 (480-520) nm
- **Temperature**: 37°C
- **Cuvette**: 1 cm light path
- **Method**: endpoint (increasing)

**Calculation using calibration**

\[ \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}} \]

A = absorbance, C = concentration

**Quality control**

Quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**PERFORMANCES DATA**

The following data were obtained using the Hitachi 717 analyzer (37°C).

**Linearity**

Relationship of absorbance vs concentration is linear up to chloride ion concentration of 135 mmol/l (479 mg/dl)

**Sensitivity**

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.25 mmol/l (0.89 mg/dl) chloride concentration at 492 nm.

**Precision**

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>Sample</th>
<th>Average concentration (mmol/l)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample I</td>
<td>111</td>
<td>2.6</td>
<td>2.35</td>
<td></td>
</tr>
<tr>
<td>sample II</td>
<td>109</td>
<td>1.3</td>
<td>1.19</td>
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</table>

<table>
<thead>
<tr>
<th>Repeatability</th>
<th>Sample</th>
<th>Average concentration (mmol/l)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
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<td>sample I</td>
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<td>1.19</td>
<td>1.42</td>
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<td>sample II</td>
<td>112</td>
<td>1.05</td>
<td>0.94</td>
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</tbody>
</table>

**Correlation**

Comparative studies were done to compare our reagent with ion-selective measurement. The results from these studies are detailed below.

Correlation coefficient: \( r=0.9903 \)

Linear regression: \( y = \frac{0.999x}{4.366} \)

(x= ion selective measurement, y= own reagent).

**Specificity**

Hemoglobin 77.5 µmol/l (500mg/dl), bilirubin 855 µmol/l (50 mg/dl), lipid 700mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 1.99 mmol/l (35mg/dl) don’t interfere with the assay up to the given levels.

**NOTE**

In the course of determination please use disposable equipments. Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

**For in vitro diagnostic use only.**

The following symbols are used on labels

- IVD  For in vitro diagnostic use
- Use by (last day of the month)
- Temperature limitation
- Batch Code
- Code

**Bibliography**