**Method:** Colorimetric, endpoint, increasing reaction.

**TEST PRINCIPLE**

This assay method is based on the following reaction sequences:

\[
6 \text{Cl}^- + 3 \text{Hg(II)Th} + 2 \text{Fe(III)Th} \rightarrow 3 \text{HgCl}_2 + 6 \text{SCN}^- + 2 \text{Fe(II)Th}
\]

Chloride ions in the sample react with mercuric thiocyanate releasing equivalent quantities of thiocyanate. Free thiocyanate ions then react with iron ions forming a red coloured complex whose absorbance at 436 nm is proportional to the chloride concentration in the sample.

**REAGENT COMPOSITION**

**COMPONENTS**

- Mercury (II) thiocyanate: 2 mmol/L
- Mercury (II) chloride: 0.8 mmol/L
- Ferric (III) nitrate: 20 mmol/L
- Nitric acid: 28 mmol/L

**REAGENT PREPARATION**

The reagent is ready to use.

**SAMPLE STABILITY AND STORAGE**

**Stability [3]:**
- at 20 – 25 °C: 7 days
- at 4 – 8 °C: 7 days
- at -20 °C: at least one year

Freeze only once! Discard contaminated specimens.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- General laboratory equipment
- **STANDARD** (not included in the kit – has to be ordered separately)
  - Concentration: 100 mmol/L
  - Storage: 2 – 25 °C
  - Stability: up to the expiration date

**MANUAL TEST PROCEDURE**

1. Pipette into test tubes:
   - Blank
   - Std./Cal.
   - Sample

2. Pipette into test tubes:
   - Reagent: 1000 µL
   - Std./Cal: 10 µL
   - Distilled water: 10 µL

3. Mix. Incubate for 5 min. at 20 – 25 °C / 37 °C. Read absorbance against reagent blank.

**CALCULATION**

\[
\text{Chloride [mmol/L]} = \frac{\Delta A \text{Sample}}{\Delta A \text{Std/Cal}} \times \text{Conc. of Std/Cal [mmol/L]}
\]

**UNIT CONVERSION**

- mmol/L = mEq/L
- mmol x 3.545 = mg/dL

**REFERENCE RANGES [1] [2] [mmol/L]**

- **Adults:** 95 – 105
- **Children:**
  - 1 – 7 days: 96 – 111
  - 7 – 30 days: 96 – 110
  - 1 – 6 months: 96 – 110
  - 6 months – 1 year: 96 – 108
  - > 1 year: 96 – 109

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

**PERFORMANCE CHARACTERISTICS**

**LINEARITY, MEASURING RANGE**

The test has been developed to determine chloride concentrations within a measuring range from 1 to 130 mmol/L. Samples with chloride ion concentrations higher than 130 mmol/L should be diluted 1+1 with distilled or deionized water and the results multiplied by 2.

**PRECISION (at 25°C)**

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>SD [mmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 20</td>
<td>Mean [mmol/L]</td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>96.5</td>
<td>1.85</td>
</tr>
<tr>
<td>Sample 2</td>
<td>110</td>
<td>3.56</td>
</tr>
<tr>
<td>Sample 3</td>
<td>117</td>
<td>2.70</td>
</tr>
</tbody>
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<td>n = 20</td>
<td>Mean [mmol/L]</td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>97.5</td>
<td>2.12</td>
</tr>
<tr>
<td>Sample 2</td>
<td>108</td>
<td>2.26</td>
</tr>
<tr>
<td>Sample 3</td>
<td>117</td>
<td>1.70</td>
</tr>
</tbody>
</table>
SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 1 mmol/L

INTERFERING SUBSTANCES
no interference up to:
Ascorbic acid  
30 mg/dL
Bilirubin  
20 mg/dL
Hemoglobin  
500 mg/dL
Triglycerides  
250 mg/dL
For further information on interfering substances refer to Young DS [5].

CALIBRATION
The assay requires the use of a Chloride Standard or a Chloride Calibrator.
We recommend the Dialab Chloride Standard or the multi calibration serum Diacal Auto. The calibration values have been made traceable to the reference method coulometry.

QUALITY CONTROL
All control sera with Chloride values determined by this method can be used.
We recommend the Dialab serum controls Diacon N (control serum with values in the normal range) and Diacon P (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION
Special applications for automated analysers can be made on request.

WASTE MANAGEMENT
Please refer to local legal requirements.

WARNINGS AND PRECAUTIONS
   H290: May be corrosive to metals.
   H411: Toxic to aquatic life with ling lasting effects.
   P 234: Keep only in original container.
   P 391: Collect spillage.
   P 501: Dispose of contents/container to hazardous or special waste collection point.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
5. For professional use only!

REFERENCES