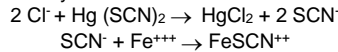


Quantitative determination of chloride ion IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate and subsequent formation of a red ferric thiocyanate complex is measured colorimetrically:



The intensity of the color formed is proportional to the chloride ion concentration in the sample^{1,2,3,4}.

CLINICAL SIGNIFICANCE

It is important clinically the determination of chloride due regulation of osmotic pressure of extra cellular fluid and to its significant role in acid-base balance. Increases in chloride ion concentration may be found in severe dehydration, excessive intake of chloride, severe renal tubular damage and in patients with cystic fibrosis.

Decrease in chloride ion concentration may be found in metabolic acidosis, loss from prolonged vomiting and chronic pyelonephritis^{2,7,8}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R (Note 4)	Mercuric thiocyanate	4 mmol/L
Mercuric thiocyanate	Ferric nitrate	40 mmol/L
	Nitric acid	45 mmol/L
CHLORIDE CAL	Chloride aqueous primary standard	125 mmol/L

PRECAUTIONS

R: H314-Causes severe skin burns and eye damage.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Reagent and standard are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 480 nm $\geq 0,15$.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 480 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment (Note 2,3).

SAMPLES

- Serum, plasma, CSF, sweat and other body fluids^{1,2}: Free of hemolysis and separated from cells as rapidly as possible. Anticoagulants such as oxalate or EDTA are not acceptable they will interfere with results.
- Urine¹: Collect 24-hour urine specimen in chloride free containers. Dilute a sample 1/2 in distilled water. Mix. Multiply results by 2 (dilution factor). Stability of the sample: Ion chloride is stable 1 week at room temperature (15-25°C), in refrigerator (2-8°C) or frozen (-20°C) temperatures.

PROCEDURE

- Assay conditions:
Wavelength: 480 (440-500) nm
Cuvette: 1 cm. light path
Temperature: 37°C / 15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette (Note 6):

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard (Note 1,5) (µL)	--	10	--
Sample (µL)	--	--	10
- Mix and incubate for 5 min at 37°C / 15-25°C.
- Read the absorbance (A) of the samples and Standard, against the Blank. The color is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A)_{\text{Sample}} - (A)_{\text{Blank}}}{(A)_{\text{Standard}} - (A)_{\text{Blank}}} \times 125 \text{ (Standard conc.)} = \text{mmol/L chloride in the sample}$$

$$\text{Urine 24 h: } \frac{(A)_{\text{Sample}} - (A)_{\text{Blank}}}{(A)_{\text{Standard}} - (A)_{\text{Blank}}} \times 125 \times \text{vol. (dL) urine/24 h} = \text{mmol/24 h chloride}$$

Conversion factor: mmol/L = mEq/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic .
If control values are found outside the defined range, check the instrument, reagent and calibration material.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

Serum or plasma:	95 - 115 mmol/L
Urine:	110 - 250 mmol/24h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,454 mmol/L to *linearity limit* of 190 mmol/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with distilled water and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mmol/L)	84,2	114	82,5	111
SD	0,81	0,62	1,07	1,87
CV (%)	0,96	0,55	1,30	1,68

Sensitivity: 1 mmol/L = 0,00471 A.

Accuracy: Results obtained using Audit Diagnostics reagents did not show systematic differences when compared with other commercial reagents. The results obtained using 50 samples were the following:

Correlation coefficient (r)²: 0,96731.

Regression equation: y=0,990x + 0,100.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis. Anticoagulants other than heparin¹.
Bilirubin up to 120 mg/L, bovine serum albumin up to 150 g/L and triglycerides up to 6 g/L did not significantly alter the assay⁴.
A list of drugs and other interfering substances with chloride determination has been reported^{5,6}.

NOTES

- CHLORIDE CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
- It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with H₂SO₄ - K₂Cr₂O₇ Solution and then thoroughly rinsed it with distilled water.
- Most of the detergents and water softening products used in the laboratories contains chelating agents. A defective rinsing will invalidate the procedure.
- Avoid the contact with metal materials.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.
- Audit Diagnostics has instruction sheets for several automatic analyzers.

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