CHLORIDE

Colorimetric determination of Chloride in biological fluids

IVD

ORDER INFORMATION

REF	Kit size
GA4330 00	4x50 ml
KL4330 00	8x60 ml
BK4330 00	2x40 ml

INDICATION

Chloride (Cl^-) is the element that has the highest extracellular concentration in the serum. Chloride plays an important role in maintaining electrolyte balance, hydration, and osmotic pressure. It is ingested through a normal diet, absorbed in the intestine, and removed from the body by excretion in urine and sweat. Excessive amounts of chloride can be lost during periods of intense perspiration. Normally elevations of chloride will be accompanied by elevations of sodium. Increased chloride level is found in dehydration, certain types of renal tubular acidosis, and hyperventilation. Decreased levels are found in uncontrolled diabetes, metabolic acidosis and Addison' disease.

METHOD PRINCIPLE

The method is based on the shift of the thiocyanate ion from mercuric thiocyanate by Chloride ion and subsequent reaction of SCN^- with iron ion yielding a red coloured compound according to the following reaction:

$Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + 2(SCN)^-$
$3(SCN)^- + Fe^{3+} \rightarrow Fe(SCN)_3$

Developed colour intensity is directly proportional to the Chloride ions present in the sample.

COMPOSITION

REAGENT A:

Mercuric thiocyanate	2 mmol/l
Iron nitrate	30 mmol/l
Nitric acid	40 mmol/l

STANDARD:	1x5 ml
Chloride	100 mEq/l

Verified against NIST reference material.

Preparation
Reagents are liquids ready to use.

Storage and stability
Store at 15-25 °C, in the dark.
Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light.
The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

SAMPLES

Serum, plasma, urine 24h or liquor.
Immediately separate the serum from cells.
Dilute urine 1:2 with distilled water.
Stable 7 days at 2-15 °C.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use controls with known Chloride concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H_2O	5 µl		
Standard	-	5 µl	-
Sample	-	-	5 µl
Mix and incubate for **10 minutes** at **room temperature** (20-25 °C). Read the absorbance (A) of the standard and samples at **470 (460-480) nm** against Blank. Colour is stable for 120 minutes protected from light.			

Note: reaction volumes can be proportionally changed.

CALCULATION OF RESULTS

Utilize the following formula:

Serum, plasma, liquor:

$$\text{Chloride, mEq/l} = \frac{\text{A sample}}{\text{A standard}} \times 100$$

Urine:

$$\text{Chloride, mEq/24h} = \frac{\text{A sample}}{\text{A standard}} \times 200 \times \text{l/24h}$$

REFERENCE VALUES

Serum	95÷103 mEq/l
Urine 24h	140÷250 mEq/l
Liquor	118÷132 mEq/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of two controls at different Chloride concentration. The obtained results are reported in the following tables:

		Within-run		Between-run	
Sample	**Mean (mEq/l)**	**SD**	**%CV**	**SD**	**%CV**
Level 1	123	3.78	3.1	5.11	4.1
Level 2	142	3.29	2.3	9.08	6.4

Linearity
The assay is linear up to 200 mEq/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 1 mEq/l.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

y = 1.001x + 0.5676 mEq/l r = 0.9699

Interferences

Hemoglobin	> 500 mg/dl
Bilirubin	> 20 mg/dl
Triglycerides	> 500 mg/dl

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY
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