Atlas Medical

Chloride
Thiocyanate-Hg. Colorimetric
Quantitative determination of chloride ion

IVD *For in -vitro diagnostic and professional use only.*

Store at 2-8°C

CE

INTENDED USE
For the measurement of chloride ion concentration in human body fluids.

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

PRINCIPLE
The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate and subsequent formation of a red ferric thiocyanate complex is measured calorimetrically.

$$2\ Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2\ SCN^-$$
$$SCN^- + Fe^{+++} \longrightarrow FeSCN^{++}$$

The intensity of the color formed is proportional to the chloride ion concentration in the sample.

REAGENTS

R Mercuric-Thiocyanate (Chloride Reagent)	Mercuric thiocyanate	4 mmol/L
	Ferric nitrate	40 mmol/L
	Mercuric nitrate	2 mmol/L
	Nitric acid	45 mmol/L
Chloride standard	Chloride aqueous primary standard 125 mmol/L	

PRECAUTIONS
- Mercury(II) thiocyanate:Harmful(Xn):Hamrful by inhalation, in contact with skin and if sw allowed.
- Keep away from food, drink and animal feeding stuffs.
- After contact with skin, wash immediately with plenty of water.
- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
- This material and its container must be disposed of as hazardous.
- Avoid release to the environment Refer to special instructions/ safety data sheets.

PREPARATION:
- All the reagents 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 ≥0.15.

EQUIPMENTS NEEDED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 480 nm.
- Matched cuvettes 1.0 cm light Path.
- General laboratory equipment.

SAMPLES
- Serum, plasma: 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.
- Urine: 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
1. Assay conditions:

Wavelength:.....480 (440-500) nm
Cuvette:.....I cm. light path
Temperature:.....37°C /15-25°C

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	**Standard**	**Sample**
R (ml)	1.0	1.0	1.0
Standard (µl)	--	10	--
Sample (µl)	--	--	10

4. Mix and incubate for 5 min at 37°C /15-25°C.
5. Read the absorbance (A) of the samples and Standard, against the Blank the color is stable for at least 30 minutes.

CALCULATIONS
$\frac{(A)\ Sample\ -(A)\ Blank}{(A)Standard-(A)Blank}$ x 125 (Stand.conc.)=mmol/L (chloride in the sample)

Urine 24 h:
$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank}$ x 125 x vol. (dL) urine/24h =mmol/24 h (chloride in the sample)

Conversion factor: mmol/L = mEq/L.

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

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

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 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 heparin.
- 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 assay.
- A list of drugs and other interfering substances with chloride determination has been reported by Young.

NOTES

1. It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with H_2SO_4 - $K_2Cr_2O_7$ Solution and then thoroughly rinsed it with distilled water.
2. Most of the detergents and water softening products used in the laboratories contains chelating agents. A defective rinsing will invalidate the procedure.
3. Avoid the contact with metal materials.
4. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
5. Use clean disposable pipette tips for its dispensation.
6. Chloride standard: proceed carefully with this product because due to it's nature ,easily it can get contaminated.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry