CHLORIDE CE

REF 1116005
2 x 50 mL

CONTENTS
R1. Reagent 2 x 50 mL
CAL. Standard 1 x 3 mL

For *in vitro* diagnostic use only

CHLORIDE
THIOCYANATE
Colorimetric method
ENDPOINT

PRINCIPLE

Chloride ions in the sample quantitatively displaces thiocyanate from mercuric thiocyanate. Liberated thiocyanate ion reacts with ferric ion forming a red ferric-thiocyanate complex proportional to the concentration of chloride present in the sample.[1,2]

$$2\ Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2\ (SCN)^-$$

$$3\ (SCN)^- + Fe^{3+} \longrightarrow Fe(SCN)_3$$

REAGENT COMPOSITION

R1 **Thiocyanate reagent**. Mercuric thiocyanate 2 mmol/L, mercuric nitrate 0.1 mmol/L, iron nitrate 30 mmol/L, HNO_3 45 mmol/L. (see Notes). $\mathbf{X_n}$

CAL **Chloride / Phosphorus standard.** Chloride 100 mEq/L / Phosphorus 5 mg/dL.
Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

STORAGE AND STABILITY

Store at 2-30ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 470 nm > 0.050 in 1cm cuvette.

REAGENT PREPARATION

The Reagent and Standard are ready-to-use.

SAMPLES

Serum, heparinized plasma and CSF.

The sera are stable in capped tubes for at least 4 hours at room temperature, 2 days refrigerated (4-8ºC) and several months frozen (-20ºC).
Cerebrospinal fluid should be collected in three sterile tubes, with samples from the first or second tube used for chloride determinations.

INTERFERENCES

- Lipemia (intralipid >1 g/L) may affect the results.
- Bilirubin (40 mg/dL) does not interfere.
- Hemoglobin (12 g/L) does not interfere.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 470 ± 10 nm.
- Constant temperature incubator set at 37ºC (optional).
- Pipettes with disposable plastic tips to measure reagents and samples.
- Disposable plastic tubes for the tests.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled test tubes:

TUBES	Blank	Sample	CAL. Standard
R1.Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
CAL.Standard	–	–	10 µL

3. Mix gently by inversion one or two times. *Do not shake or stir vigorously.*
4. Incubate the mixture for 5 or 10 minutes at a selected constant temperature between 25-37ºC (see Notes).
5. Read the absorbance (A) of the samples and the standard at 470 ± 10 nm against the reagent blank.

The color is stable for about 2 hours, at room temperature, protected from light.

CALCULATIONS

Serum, plasma

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard = mEq/L\ (mmol/L)\ chloride$$

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Samples with concentrations higher than 125 mEq/L (125 mmol/L) should be diluted 1:2 with distilled water and assayed again. Multiply the results by 2.
Concentrations less than 75 mmol/L, should be brought within linear range by increasing the amount of serum used.

REFERENCE VALUES[2]

Serum, plasma	98 - 111 mEq/L (98 - 111 mmol/L)
CSF	120 - 130 mEq/L (120 - 130 mmol/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of chloride. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of chloride. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Sodium and chloride represent the majority of the osmotically active constituents of plasma. As a result, chloride is significantly involved in maintenance of water distribution, osmotic pressure, and anion-cation balance in the extracellular fluid compartment.

Hypochloremia (decreased plasma Cl^- concentration) is observed in salt-losing nephritis as associated with chronic pyelonephritis. In Addison´s disease, Cl^- levels as well as Na^+ levels may drop significantly during the Addisonian crisis and in certain types of metabolic acidosis (e.g., diabetic ketoacidosis and renal failure) and aldosteronism. In metabolic alkalosis, plasma levels of Cl^- tend to fall while HCO_3^- levels increase.

Hyperchloremia (increased plasma Cl^- concentration) occurs with dehydration, renal tubular acidosis, diabetes insipidus, acute renal failure, adrenocortical hyperfunction and metabolic acidosis. Extremely high dietary in take of salt and overtreatment with saline solutions are also causes of hyperchloremia.

ANALYTICAL PERFORMANCE

- ***Linearity :*** Between 70 and 125 mmol/L
- ***Precision:***

mmol/L	Intraserial			Interserial		
Media	75	100	120	75	101	121
DE	1.3	2.2	1.7	1.8	2.5	1.7
CV%	1.7	2.2	1.4	2.4	2.5	1.4
N	10	10	10	10	10	10

- ***Sensitivity :*** 0.005A / mmol/L chloride.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:

N = 20 r = 0.993 y = 1.05x - 3.1

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

- The reagent is harmful if swallowed. **Do not pipette by mouth**. In case of accident or if you feel unwell, seek medical advice immediately.
- A 5-min incubation period is satisfactory when the incubation and assay are performed at 37ºC. A 10-min incubation period is recommended when the incubation and assay are performed at room temperature.
- For optimum results, the glassware used for the chloride procedure should be scrupulously clean. It may be found convenient to acid wash the material (H_2SO_4-$K_2Cr_2O_7$) and then thoroughly rinse it with distilled water.
- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

1. Schoenfeld R.G., and Lewellen Clin. Chem. 10 : 533 (1964).
2. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

B1116-2/0901
R1.ing

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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