CHLORIDES Liquiform
Insert

Ref.: 115

Intended use . Colorimetric end-point reaction system for quantitative determination of chloride ion concentration in serum, plasma (heparin), urine, and cerebrospinal fluid by an end point reaction.

Professional use.

[Only for in vitro diagnostic use.]

Test principle[1,2] . Chloride ions in the sample react with mercury thiocyanide, yielding mercuric chloride and thiocyanide ions. Thiocyanide ions react with ferric ions, yielding ferric thiocyanide, according to the equation below:

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

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

Ferric thiocyanide is yellow and its color intensity, which is measured between 450 and 505 nm, is proportional to the chloride ion concentration in the sample.

Summary[1,2,8,9] . The Chloride method from Labtest is a simple and fast procedure for colorimetric determination of chloride ions in biologic fluids.

The system presents a linear response up to 130 mEq/L of chloride ions.

Repeatability and reproducibility data show that the method yields results which meet the desirable specifications for total error, based on CLIA specifications.

The method does not suffer interference of bilirubin in concentrations up to 38 mg/dL, hemoglobin up to 180 mg/dL, and triglycerides up to 1000 mg/dL, exhibiting excellent correlation with chloride determination by Ion-Selective Electrode (ISE).

The method is easily applicable to automated analyzers capable of accurately measuring an end-point reaction between 450 and 505 nm. The Chlorides Liquiform system can also be applied to manual use with semi-automated equipment.

Methodology[2] . Mercury Thiocyanide.

Reagents

1. R 1 - Reagent 1 - Store at 2 - 30 ºC.
Handle carefully: toxic reagent. Do not pipette by mouth. Contains mercury thiocyanide 2.0 mmol/L, mercury chloride >0.8 mmol/L, ferric nitrate >20 mmol/L, nitric acid 28 mmol/L, and stabilizer.

2. CAL - Standard - Chloride 100 mEq/L - Store at 2 - 30 ºC.
After manipulation, store tightly closed to avoid evaporation.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.
In order to avoid evaporation of the Standard, keep the bottle tightly closed.

Deterioration . Microbial or chemical contamination may decrease reagent stability.

Precautions and warnings

Do not use Reagent 1 or Standard if they show signs of contamination (turbidity, precipitation, or color alteration).

The usual security cares should be applied to the reagent handling. Reagent 1 contains mercury thiocyanide, which is toxic.

Materials required not provided

1. Photometer capable of measuring absorbance at 450 - 505 nm.
2. Pipets to measure reagents and samples.
3. Timer.

Specimen collection and preparation[3]

Use serum, plasma (heparin), urine, or cerebrospinal fluid.

Important . Plasma collected with citrate or EDTA yields falsely lower results.

To avoid chloride diffusion to erythrocytes, the plasma or serum sample must be separated up to 1 hour after blood collection. The analyte is stable in serum or plasma for 7 days at 15 - 25 ºC, 4 weeks at 2 - 8 ºC, and several months at -10 ºC.

To determine the chloride concentration in cerebrospinal fluid or urine, use centrifuged samples.

To determine the chloride concentration in sweat, it is recommended to use the Ion-Selective Electrode (ISE) methodology.

A Standard Operating Procedure (SOP) must be created to establish adequate procedures for sample collection, preparation, and storage. The errors due to bad sampling can be more damaging than the ones which may occur during the analytical procedure.

Disposal of all waste material should be in accordance with local guidelines.

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Interference[1,9]

Bilirubin up to 38 mg/dL, hemoglobin up to 180 mg/dL, and triglycerides up to 1000 mg/dL do not interfere significantly.
Triglycerides above 1000 mg/dL yield falsely elevated results. In such cases, the sample blank is not applicable.

Manual procedure

See linearity and observation[1].

Serum, plasma (heparin), or cerebrospinal fluid.

Urine: dilute the sample 1:2 with deionized water. Multiply the result obtained by 2.

Set up three tubes and proceed as follows:

	Blank	Unknown	Standard
Sample	-----	0.01 mL	-----
Standard	-----	-----	0.01 mL
Reagent 1	1.0 mL	1.0 mL	1.0 mL

Homogenize the tubes and incubate them at room temperature for 2 minutes. Determine the absorbances for unknown and standard at 450 nm (450 - 505 nm), determining the zero point with the blank. The reaction color is stable for 2 hours.

The suggested procedure is adequate for photometers with minimum reading solution volume equal to or less than 1 mL. The need of volume adjustment must be established. Sample and reagent volumes can be modified proportionally without impairing the method performance, and the calculation procedure remains the same. If a volume reduction is necessary, the minimum volume for photometric reading must be observed. Sample volumes lower than 10 micro liters are critical in manual methodologies and must be used cautiously, since they tend to raise the measurement imprecision.

Calculations . See linearity

$$\text{Chlorides (mEq/L)} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times 100$$

The result can also be obtained with the calibration factor:

$$\text{Calibration Factor} = \frac{100}{\text{Standard Absorbance}}$$

Chlorides (mEq/L) = Test Absorbance x Calibration Factor

Example

The data below are merely for illustrative purposes and must not be used to calculate real laboratory results.

Test Absorbance: 0.562
Standard Absorbance: 0.524

$$\text{Chlorides (mEq/L)} = \frac{0.562}{0.524} \times 100 = 107$$

or,

$$\text{Calibration Factor} = \frac{100}{0.524} = 190$$

Chlorides (mEq/L) = 0.562 x 190 = 107

Urine (mEq/24hours) = mEq/L x volume (L).

Calibration

Manual calibrations
Perform a new calibration after reagent lot change or when the internal quality control indicates.

Automatic Systems
Blank of reagents: Deionized water
Standards: Calibra Series (Labtest calibrators for automated systems).

Calibration frequency
Two point calibration after reagent lot change;
Two point calibration when the internal quality control indicates.

Operating range . The measurement result is linear up to 130mEq/L. For larger values, dilute the sample with distilled or deionized water, perform a new measurement and multiply the result obtained by the dilution factor.

Internal Quality control[4,6,7,8] . The clinical laboratory must keep an internal quality control program, defining clearly all applicable regulations, objectives, procedures, quality specification criteria, tolerance limits, corrective measures, and registration of activities. Control materials must be used to monitor the measurement imprecision and calibration deviation. It is recommended that the CLIA specifications for coefficient of variation and total error be followed.

For quality control use Qualitrol H Level 1 and Qualitrol H Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Serum or plasma (all ages) . 98 - 110 mEq/L.
Urine . 110 - 250 mEq/24 hours.
CSF . 113 - 127 mEq/L.

Conversion: Conventional units (mEq/L) x 1.0 = SI Units (mmol/L)

Performance characteristics[7,8,9]

Recovery studies . In a sample with chloride concentration of 84 mEq/L different quantities of chloride were added. Subsequent analyses provided recoveries ranging from 100 to 103%.

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Method comparison . The proposed method was compared to the ISE methodology, using samples with values between 90 and 140 mEq/L. The method comparison produced the regression equation y = 0.7997x + 19.125 and a correlation coefficient (r) of 0.998. The total systematic error (constant and proportional) verified at the 94 mEq/L decision level was equal to 0.32%, and it was equal to 2.9% at the 112 mEq/L decision level.

Imprecision - Within run

	N	Mean (mEq/L)	SD (mEq/L)	%CV
Sample 1	20	94	0.39	0.42
Sample 2	20	112	0.70	0.63

Imprecision - Run-to-run

	N	Mean (mEq/L)	SD (mEq/L)	%CV
Sample 1	20	94	0.97	1.03
Sample 2	20	112	1.17	1.04

The estimated total error (random error + systematic error) is 2.3% at the 94 mEq/L decision level and 4.9% at the 112 mEq/L decision level. These results indicate that the method meets the desirable specification for total error (<±5%) based on CLIA specifications.

Analytical sensitivity . Detection limit: 0.44 mEq/L. The detection limit represents the lowest measurable chloride concentration that can be distinguished from zero. It is calculated as three standard deviations from the mean of 20 replicates of one sample without chloride.

Effects of matrix dilution . Three samples with concentrations equal to 130, 118 and 105 mEq/L were used to evaluate the system response in the dilution of the matrix with deionized water. Using dilution factor of 2 and 4, recoveries between 101 and 105% were found.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult Young DS. Effects of Drugs on Clinical Laboratory Tests, 3rd Edition, Washington: AACC Press, 1990 to review physiopathological sources and drug interferences in results and methodologies.

References

1. Burtis C.A, Ashwood E.R. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th edition, Elsevier Saunders Company 2006;989-990.
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3. Chronolab - "The Quality of Diagnostic samples" - www.diagnosticsample.com. Accessed on 25/02/2010.
4. Inmetro - Boas Práticas de Laboratório Clínico e Listas de Verificação para Avaliação, Qualitymark eds, Rio de Janeiro, 1997.
5. Toffaletti, J.G. "Electrolytes" In: Bishop, M.L., Duben-Engelkirk, J.L., Fody, E. P. Clinical Chemistry: principles, procedures, correlations. 3 ed. Philadelphia. Lippincott, 1996. p 255-278.
6. Tonks DB. Quality Control in Clinical Laboratories, Warner-Chilcott Laboratories, Diagnostic Reagents Division, Scarborough, Canada, 1972.
7. Westgard JO, Barry PL, Hunt MR, Groth T.1981;27:493-501.
8. CLIA Requirements for Analytical Quality - Westgard QC - Federal Register February 28, 1992;57(40):7002-186 - http://www.westgard.com/clia.htm. Accessed on 18/02/2010.
9. Labtest: data on file.

Presentation

Product	Reference	Contents	
Chlorides Liquiform	115-1/50	R 1	1 X 50 mL
		CAL	1 X 3 mL

Application procedures using Chlorides Liquiform are available **for various automated systems.**

The number of tests in automated systems **depends on the programmed parameters.**

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label provided that the procedures and storage conditions indicated on the label and in this insert have been followed correctly.

CE

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

 **Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests	 **Risco biológico** Riesgo biológico Biological risk
 **Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	 **Marca CE** Marcado CE CE Mark
 **Material Calibrador** Material Calibrador Calibrator Material	 **Tóxico** Tóxico Poison
 **Material Calibrador** Material Calibrador Calibrator Material	 **Reagente** Reactivo Reagent
 **Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)	 **Fabricado por** Elaborado por Manufactured by
 **Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	 **Número do lote** Denominación de lote Batch code
 **Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	 **Controle** Control Control
 **Número do catálogo** Número de catálogo Catalog Number	 **Controle negativo** Control negativo Negative control
 **Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	 **Controle positivo** Control positivo Positive control
 **Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	 **Controle** Control Control
 **Liofilizado** Liofilizado Lyophilized	 **Corrosivo** Corrosivo Corrosive
 **Período após abertura** Período post-abertura Period after-opening	 **Uso veterinário** Uso veterinario Veterinary use
 **Instalar até** Instalar hasta Install before	

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