CA ARSENAZO Liquiform

Instruction for use

Ref.: 95

Intended use . End point reaction system for determination of calcium in blood and urine.

Professional use.

[For in vitro diagnostic use.]

Test principle . Calcium reacts with arsenazo III in a solution a little acid yielding the blue colored complex calcium-arsenazo III whose intensity is proportional to the calcium concentration in sample. The reaction product absorbance must be measured at 600 nm or 660 nm.

Summary . The photometric measures of calcium in blood and urine by direct methods are based on the complex formation with organic molecules. Among the compounds that react with calcium, arsenazo III is used very often in clinical laboratory assays. During the data collected by the College of American Pathologists in 2002, 39% participants used these reagents containing arsenazo III complex.

The reagent is available in liquid form in only one reagent, what makes easy its application and use. Labtest system does not suffer interference of bilirubin up to 20 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 1100 mg/dL when the product absorbance is measured at 660 nm.

The method is not interfered by plasmatic proteins and the magnesium effect is eliminated by adding 8-hydroxyquinoline, what makes this test one of the most specific for calcium determination.

The system is easily applied to most automated and semi-automated equipments which are able to measure end point reaction at 600 or 660 nm.

Methodology . Arsenazo III

Reagents

1. R 1 - Reagent 1 - Store at 2-8ºC.

Reagent label bears expiration date.

Buffer pH 6.8 (100 mmol/L), arsenazo III (≤0.2 mmol/L), 8-hydroxyquinoline (≥5 mmol/L) sodium azide (<0.1 %), and surfactants.

2. CAL - Standard - Store at 2-30ºC.

Reagent label bears expiration date. Calcium (10 mg/dL), sodium azide (<0.1 %).

Precautions and warnings

For in vitro diagnostic use.

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

The usual security cares should be applied on the reagent handling.

The Reagent 1 and Standard contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to 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 reagents stability.

Sample

Use serum or plasma (heparin) or urine.

Due the rise in the erythrocytes permeability to calcium, separate the serum or plasma within one hour after blood collection. Calcium is reportedly stable for about 2 weeks at 2-8ºC and 4 weeks at - 10ºC.

Urine should be collected into a bottle containing 20 mL of 50% (v/v) Hcl 6M and the sample must be homogenized during the collection.

If the 24 hour urine was not acidified during the collection, add 20 mL of HCl 6M, homogenize and wait 60 minutes to use an aliquot for the assay. The previously acidified sample must be homogenized before using it.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Physical exercises increase 3-5% calcium values.

Calcium concentration decreases about 10% during the pregnancy.

Calcium values are slightly decreased in chronic alcoholism and slightly increased after hemodialysis.

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Citrate, oxalate, fluoride or EDTA plasma provides false decreased results due the stable calcium complex formation.

Bilirubin up to 20 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 1100 mg/dL do not interfere significantly when the absorbance of the reaction product is measured at 660 nm.
Hemoglobin values over than 60 mg/dL yield significant interferences when the reaction product is measured at 600 nm.

Materials required not provided

1. Photometer capable of measuring absorbance at 600 or 660 nm.
2. Pipettes to measure reagents and samples.

Manual procedure

See notes 1, 2 and 3.

Set up two tubes ("Unknown" and "Standard") and proceed as follows:

	Unknown	Standard
Reagent 1	1.0 mL	1.0 mL

Adjust the photometer at 600 nm (600-610 nm) or 660 nm (650 to 660 nm).

Take the "Unknown" tube and zero the instrument. Next, don't change any controls, add 0.01 mL of sample into this tube. Mix well and measure the absorbance ($A_{Unknown}$).

Take the "Standard" tube and zero the instrument. Next, don't change any controls, add 0.01 mL of standard into this tube. Mix well and measure the absorbance ($A_{Standard}$).

This procedure eliminates the interferences caused by traces of calcium on the tubes.

Calibration . The standard is traceable to NIST SRM 915.

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

Automatic systems
Blank of reagents: water or 0.85% NaCl;
Standards: Calibra Series (Labtest calibrator for automated systems), which are traceable to NIST SRM 915.

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

Quality control . 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.

Calculations

$$\text{Calcium (mg/dL)} = \frac{A_{unknown}}{A_{standard}} \times 10$$

Due the great reproductive results of the assays system, it is possible to use the factor method:

$$\text{Calibration factor} = \frac{10}{A_{standard}}$$

$$\text{Calcium (mg/dL)} = A_{Unknown} \times \text{Factor}$$

$$\text{Urinary calcium (mg/24 hours)} = \frac{\text{urinary calcium (mg/dL)} \times \text{urinary volume (mL)}}{100}$$

Calcium by glomerular filtration volume . Urinary calcium (UCa) measured in a urine sample may be related to the volume of glomerular filtration (GF). This relation is an alternative for obtaining values of calciuria when a 24 hour sample is not available. Collect urine and blood sample after 8 hour fasting. Measure the calcium and creatinine concentrations in urine and creatinine in serum, and calculate as follows:

$$\text{Calcium (mg/100mL of GF)} = \frac{\text{UCa (mg/dL)} \times \text{serum Creatinine (mg/dL)}}{\text{Urine Creatinine (mg/dL)}}$$

Ionized calcium (Ica)

The ionized calcium (ICa) can be determined using the dosages of serum calcium, total protein and albumin.

$$\text{ICa (mg/dL)} = \frac{6 \times Ca - \frac{(0.19 \times P) + A}{3}}{(0.19 \times P) + A + 6}$$

Ca = Serum Calcium (mg/dL)
P = Total Protein (g/dL)
A = Albumin (g/dL)

Measurement/reportable range

Up to 17 mg/dL.

If calcium concentration exceeds 17 mg/dL, the sample must be diluted 1:2 or 1:4 with 0.85% NaCl 150 mmol/L. Multiply the result by the appropriate dilution factor.

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

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Serum
Calcium

Children[8,9]	
Age	mg/dL
Umbilical Cord	8.2 - 11.2
Premature	6.2 - 11.0
0 - 10 days	7.6 - 10.4
Suckling	9.0 - 11.0
2 - 12 years old	8.8 - 10.8

Calcium

Adults	mg/dL
	8.8 - 11.0

Ionized calcium (mg/dL)

1 a 18 years old[8,9]	4.80 - 5.52
Adults	4.60 - 5.40

Urine . 5 a 40 mg / 24 hours in exempt calcium diet. Up to 200 mg / 24 hours in restricted calcium diet (500 mg / 24 hours). Up to 300 mg / 24 hours in unrestricted calcium diet.

Urine sample . < 0.16 mg/100mL GF

Conversion . Conventional Unit (mg/dL) x 0.25 = Unit IS (mmol/L).

Performance characteristics[10]

Recovery studies . In three samples with calcium concentrations of 7.0, 11.0 and 13.0 mg/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 90 to 92%. The mean proportional systematic error at 11 mg/dL decision level was 0.96 mg/dL or 8.7 %.

Method comparison . A group of 60 samples were assayed by the proposed method and a similar technique. The calcium values ranged from 2.7 - 17.6 mg/dL. The comparisons yielded a correlation coefficient of 0.999 and regression equation was y = 1.001x - 0.071. The mean total systematic error (proportional and constant) at 11.0 mg/dL decision level was 0.088 mg/dL or 0.8 %.

Imprecision - Within run

	N	Mean (mg/dL)	SD (mg/dL)	%CV
Sample 1	20	8.4	0.11	1.29
Sample 2	20	11.8	0.15	1.31
Sample 3	20	14.3	0.16	1.12

Imprecision - Run-to-run

	N	Mean (mg/dL)	SD (mg/dL)	%CV
Sample 1	20	8.4	0.16	1.90
Sample 2	20	11.8	0.17	1.49
Sample 3	20	14.3	0.21	1.44

Analytical sensitivity . Detection limit: 0.083 mg/dL. The detection limit represents the lowest measurable calcium concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without calcium.

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 microsiems/cm and silicates concentration must be <0.1mg/L.

3. The use of ionic detergent for cleaning materials may be a source of calcium contamination.

4. It is suggested to consult "www.fxol.org" in order to review physiopathological source and drugs interference in results and methodology.

References

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10. Labtest: data on file.

Presentation

Product	Reference	Contents	
Ca Arsenazo Liquiform	95-2/50	R 1	2 x 50 mL
		CAL	1 x 5 mL
Ca Arsenazo Liquiform Linha Audmax i	95-3/40	R 1	3 x 40 mL
		CAL	1 x 5 mL
Ca Arsernazo Liquiform Linha CS 400/800	95-4/43	R 1	4 x 43 mL
		CAL	1 x 5 mL

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

The number of tests in automated systems **depends of 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 since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

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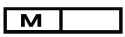
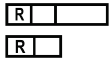
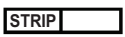
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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with IVD devices

	**Conteúdo suficiente para < n > ensaios** Contenido suficiente para < n > ensayos Sufficient content for < n > trials		**Risco biológico** Riesgo biológico Biological risk
	**Prazo de validade (aaaa-mm-dd ou mm/aaaa)** Fecha de expiración (aaaa-mm-dd o mm/aaaa) Expiration date (yyyy-mm-dd or mm/yyyy)		**Corrosivo** Corrosivo Corrosive
	**Limite de temperatura (conservar a)** Temperatura límite (conservar a) Temperature limit (store at)		**Tóxico** Tóxico Poison
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Marca CE** Marcado CE CE Mark
	**Carcinogênico/mutagênico e/ou sensibilizante à respiração** Carcinogénico/mutagénico y/o sensibilizante respiratorio Carcinogenic/mutagenic and/or respiratory sensitizer		**Atenção** Atención Attention
	**Tóxico para os organismos aquáticos** Tóxico para los organismos acuáticos Toxic for aquatic organisms		**Data de fabricação** Fecha de fabricación Date of manufacture
	**Gases/líquidos comburentes** Gases/líquidos oxidantes Oxidizing gases/liquids		**Fabricante** Fabricante Manufacturer
	**Substância inflamável** Sustancia inflamable Flammable substance		**Liofilizado** Liofilizado Lyophilized
	**Período após abertura** Período post-abertura Period after-opening		**Número do lote** Denominación de lote Batch code
	**Produto de uso único** Producto de un solo uso Single use product		**Número do catálogo** Número de catálogo Catalog Number
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Material Calibrador/Padrão** Material Calibrador/Estándar Calibrator/Standard Material		**Controle negativo** Control negativo Negative control
	**Dispositivo médico de diagnóstico in vitro** Dispositivo médico para diagnóstico in vitro In vitro diagnostic medical device		**Controle positivo** Control positivo Positive control
	**Reagente contendo microparticulas** Reactivo con microparticulas Reagent with microparticles		**Reagente** Reactivo Reagent
	**Identificador único do dispositivo** Identificador único del dispositivo Unique device identifier		**Pré-Tratamento** Pretratamiento Pre-Treatment
	**Cartucho contendo reagente** Cartucho que contiene reactivo Cartridge containing reagent		**Instalar até** Instalar hasta Install before

Ref.: 031125

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