CALCIUM AIII LS "650" Mono

IVD

Quantitative determination of Calcium in Serum, Plasma or Urine. Colorimetric method (Arsenazo III).

REF **CC1052** R1: 4x60 mL + R2:1x3 mL (standard)

METHOD AND PRINCIPLE

With Arsenazo III and at neutral pH, Calcium produces a blue complex, whose optical absorbance, proportional to the Calcium concentration in the sample, is photometrically measurable at 650 nm.
The use of a calibrator with a known title allows to calculate the concentration in unknown samples.

CLINICAL SIGNIFICANCE

Calcium exists in the blood in three forms: ionized (13%), complexed (47%) and bound to protein, mainly albumin (40%). When calcium determinations are performed, the total calcium concentration is determined regardless of the amount of calcium present in each form.
A depressed concentration of total calcium can be due to hypo-proteinemia, but the concentration of physiologically active (ionized) calcium in such case may be normal. For this reason, a protein determination should accompany each calcium analysis so that the calcium value can be interpreted properly. Depressed serum calcium levels usually accompany hypoparathyroidism, some bone diseases, certain kidney diseases, and low protein levels. Elevated serum calcium levels occur in hyperparathyroidism, vitamin-D poisoning, and sarcoidosis. The plasma level in calcium is greatly affected by the plasma level of inorganic phosphate. In most cases, there is an inverse relationship between calcium and inorganic phosphate. Conditions associated with hypercalcemia, such as primary hyperparathyroidism are usually associated with hypophosphatemia; the opposite is true as well.
Urine calcium excretion parallels the serum calcium level. Large amounts of calcium are excreted in the urine in hyperparathyroidism, metabolic acidosis, renal tubular insufficiency, and multiple myeloma and bone malignancies.

REAGENT COMPOSITION

Reagent (R1)

Imidazole buffer, pH 6.5	70 mmol/L
Arsenazo III	120 µmol/L
Detergents	2 %
Sodium Azide	0,95 g/dL

Reagent (R2)

Standard (Calcium):	see value on label

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light.
Do not freeze the reagents.
Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera.
After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

SPECIMEN

Serum, heparin plasma, urine. Do not use EDTA plasma.
The 24h Urine must be acidified with 10 mL of concentrated hydrochloric acid. The morning urine should be acidified with a few drops of concentrated hydrochloric acid.
The urine sample should be diluted 1: 2 (1 + 1) with distilled water.
Stability in serum /plasma:
7 days at 15° - 25 °C, 3 weeks at 2°- 8 °C, 3 months at -20 °C.
Stability in urine:
2 days at 15°- 25 °C, 4 days at 2°- 8°C, 3 weeks at -20 °C.
Discard contaminated samples.

PROCEDURE

Wavelength:	650 nm (630-700)
Temperature:	37 °C
Measurement:	against Reagent Blank

Pipette as follow:

R1 Reagent	1000 µL
Sample, Std / Cal	10 µL

Mix, incubate for 5 minutes and read absorbance against Reagent Blank within 60'.

CALCULATION

Serum or Plasma:

$$\text{Calcium} = \frac{\text{Abs Sample}}{\text{Abs Std/Cal}} \times \text{Concentration Std/Cal}$$

Urine:
Calculate as for the serum/plasma and multiply the result by 2 (initial sample dilution)
Calcium [mg/24h] = Urinary Calcium (mg/dL) x Urine Volume 24 h (dL)
Calcium [mmol/24h] = Urinary Calcium (mmol/L) x Urine Volume 24 h (L)

Conversion Factor: Calcium [mg/dL] x 0.2495 = Calcium [mmol/L]

CALIBRATION WITH CALIBRATOR

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.
As an alternative to the standard included in the package, it is possible to use **MTD Diagnostics Calibrator:**

Chemistry Multicalibrator - REF CAL1010 (10x3 mL)

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Chemistry Control N - REF CNN1010 10 x 5 mL (Level 1)
Chemistry Control P - REF CNP1020 10 x 5 mL (Level 2)

EXPECTED VALUES

Serum / Plasma:

New born	8.0 - 13.0 mg/dL
Children	8.5 - 12.0 mg/dL
Adults	8.5 - 10.5 mg/dL

Urine:

Women	< 250 mg/24 h
Men	< 300 mg/24 h

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 8.79; S.D. = 0.09; CV = 1.02%
High Level: Samples = 20; Average = 14.0; S.D. = 0.24; CV = 1,71%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y=1.02x-0,20 - r=0.999

SENSITIVITY: 0.10 mg/dL

LINEARITY: 0.10 - 20 mg/dL

SPECIFICITY / INTERFERENCES

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, haemoglobin up to 500 mg/dL, lipemia up to 2.000 mg/dL triglycerides and magnesium up to 15 mg/dL.

NOTES

1. As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.
2. Traces of chelating agent, such as EDTA can prevent the formation of the coloured complex.
3. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
4. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS

R1 contains ARSENAZO III – 80 µmol/L - CAS N.: 1668-00-4.
N – T R23/25 (H331 – H301) - R50 (P410) – R53 (P413).

R23/25 (H331 – H301): Toxic by inhalation and if swallowed
R50 (H400): Very toxic to aquatic organisms.
R53 (H413): May cause longterm adverse effects in the aquatic environment.

The product does not contain any other hazardous substances or mixtures according to EC Regulation No. 1272/2008 (CLP) or their concentrations are such that they are not considered to be persistent, bioaccumulative or toxic (PBT). Therefore, it is not subject to the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / EC). Sodium Azide less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

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