CALCIUM AIII

(Arsenazo III method)

INTRODUCTION

Calcium plays an essential role in many cell functions: intracellularly in muscle contraction and glycogen metabolism, extracellularly, in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium is present in plasma in three forms: free, bound to proteins or complexed with anions as phosphate, citrate and bicarbonate. Decreased total calcium levels can be associated with diseases of the bone apparatus (especially osteoporosis), kidney diseases (especially under dialysis), defective intestinal absorption and hypoparathyroidism. Increased total calcium can be measured in hyperparathyroidism, malignant diseases with metastases and sarcoidosis. Calcium measurements also help in monitoring of calcium supplementation mainly in the prevention of osteoporosis.

METHOD PRINCIPLE

Photometric test using arsenazo III. Calcium with arsenazo III at neutral pH yields a blue colored complex, whose intensity is proportional to the calcium concentration.

KIT CONTENTS

Reagent Name	MCAA02050M	VCAA02501M
R1 - Calcium Reagent	2 X 50 ml	25 X 1 ml
R2 - Calcium Reagent	2 ml	2 ml

R2-STANDARD is Calcium standard solution: Refer standard value mentioned in the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagents when stored at **R.T** are stable upto the expiry date printed on the package. The reagents are stable for 6 weeks on board the analyzer at 2-10 °C. Protect from Contamination. Do not freeze the reagents.

CONCENTRATIONS IN THE TEST

phosphtase buffer (pH 7.5) 50 mmol/l
8-Hydroxyquinoline-5-sulfonic acid 5 mmol/l
Arsenazo III 120 µmol/l

WARNINGS AND NOTES

-Product for in vitro diagnostic use only.

-Contaminated glassware is the greatest source of error. The use of disposable plastic ware is recommended. Glassware should be soaked for a few hours in 2M HCl solution and then thoroughly rinsed with distilled water.

-The reagents contains sodium azide (0.95 g/l) as preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 650 nm, Hg 623nm (630-670 nm)
- Thermostat at 37ºC
- General laboratory equipment;

SPECIMEN

Serum, heparinized plasma free from hemolysis, 24-hours urine. Do not use EDTA plasma. Traces of chelating agent, such as EDTA can prevent the formation of the colored complex.
Serum and plasma can be stored up to 7 days at 20-25°C
Add 10 ml of concentrated HCL to 24h urine and heat the specimen to dissolve calcium oxalate. Discard contaminated specimens.
24-hours urine samples can be stored up to 2 days at 20-25°C.
Nevertheless it is recommended to perform the assay with freshly collected sample.

PROCEDURE

These reagents may be used for manual assay and in several automatic analysers. **Programme Sheets** are available on request.

Wavelength 650 nm (630 - 670 nm)
Temperature 20-25°C / 37°C
Cuvette 1 cm

Pipette into the cuvettes:

Reagent	Blank (B)	Standard (S)	Test (T)
R1 Calcium Reagent	1000 µl	1000 µl	1000 µl
Bring up the temperature of determination. Then add,			
Distilled water	10 µl		
R2 - Calcium standard		10 µl	
Sample			10 µl

Mix well, incubate for 5 min. Read the absorbance against reagent blank (RB).

CALCULATION

Calcium concentration [mg/dl]= A(T) / A(S) x Standard concentration [mg/dl]

REFERENCE VALUES

Serum / Plasma	8.5 to 10.5 mg / dl
24 hours urine	100 to 250 mg / day

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.
For Fully Automated analyzers by using multi-calibrator or calcium standard the calibration curve can plot and the same should be prepared every 6 weeks or with change of reagent lot number.

PERFORMANCE CHARACTERISTICS

-Sensitivity / Limit of Quantitation: 0.121 mg/dl (0.03mmol/l).

-Linearity: up to 25 mg/dl (6.25 mmol/l). For higher concentration of calcium dilute the sample 1+1 with 0.9% NaCl and repeat the assay. Multiply the result by di l ution factor.

-Specificity / Interferences
Haemoglobin up to 500 mg/dl, ascorbate up to 30 mg/dl, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl, and magnesium up to 15 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 192-202.
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3. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971;53: 194-8.Bauer PJ. Affinity and stochiometry of calcium binding by arsenazo III. Anal Biochem 1981; 110:61-72.
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SYSTEM PARAMETERS

Method	End Point
Wavelength	630 mm
Zero Setting	Reagent blank
Temperature Setting	25° C / 37° C
Incubation Temperature	37° C
Incubation Time	5 mins
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Standard vial
Units	mg/dl
Factor	----
Reaction Slope	Increasing
Linearity	25 mg/dl

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