



CALCIUM (ARSENAZO III Method-End Point)

Intended Use

The reagents are used for the quantitative determination of Calcium in serum or plasma. For in-vitro diagnostic use only.

Introduction

Calcium is the most abundant mineral in the human body. The average adult body contains in total approximately 1 kg, 99% in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the serum. Calcium metabolism or calcium homeostasis is the mechanism by which the body maintains adequate calcium levels. Derangements of this mechanism lead to hypercalcemia or hypocalcemia, which both can have important consequences for health.

Hypercalcemia

It may be developed in case of abnormal parathyroid gland function, malignancy, vitamin-D metabolic disorders, disorders related to high bone turnover rate like Paget's disease and renal failure.

Hypocalcemia

This may be developed in case of rickets, coeliac disease, idiopathic steatorrhea, osteomalacia and following surgical resection of small intestine.

Method

Arsenazo III method, End Point.

Principle

The calcium ions in the patient serum/plasma react with Arsenazo III to form a colored complex. The intensity of the color is directly proportional to the concentration of calcium in the sample. The concentration is measured photometrically at a wavelength of 650nm (630 – 660nm), and compared with a standard.

Reagent Composition

Reagent 1:

Arsenazo III	0.047 mmol/l
Immidazole	100 mmol/l

Reagent 2 (Working solution):

Hydrochloric acid	0.1 M
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Reagent 3:

Calcium Standard	8 mg/dl
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Precautions

Following precautions should be taken:

- All the glassware should be washed with 0.1 M HCl (Washing solution) and properly rinsed with distilled water and dried before use.
- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.

Reagent Storage and Stability

Unopened Reagent is stable till expiry mentioned on the label when stored at room temperature (under 30°C).

Standard Reagent R3 is stable till expiry mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 4 (Calcium: 12 mg/dl) & Reagent 5 (Calcium: 15 mg/dl) can be provided for linearity check with Reagent 3 (Calcium: 8 mg/dl-Standard).

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagents should be a clear solution. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Serum or heparinized plasma can be used for the testing. Other anti-coagulants like EDTA, citrate and oxalates are not suitable for the assay. Samples can be stored for 7 days at 2-8°C. Venous stasis during sample collection should be avoided.

General Assay Parameters

Mode	End Point
Wavelength 1 (nm)	650
Wavelength 2 (nm)	-
Blank with	Reagent
Sample Volume (µl)	5/10
Reagent 1 (µl)	500/1000
Incubation Time (min.)	2
Incubation Temperature (°C)	RT
Normal Low (mg/dl)	8.1
Normal High (mg/dl)	10.4
Linearity (mg/dl)	Upto 15
Standard Conc. (mg/dl)	8
Units	mg/dl

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PROCEDURE

Always use 0.1 M HCl, washed & dried test tubes before test the assay. One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

Particulars	Blank	Standard	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Dist. Water	10µl	-	-
Reagent 3	-	10 µl	-
Sample	-	-	10 µl

Mix well & incubate for 2 min at room temperature. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 650 nm.

Calculation

Calcium concentration in the sample can be calculated using the following formula:

$$\text{Calcium} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.180} \times 8.0 = 8.88 \text{ mg/dl}$$

If the calcium concentration exceeds 15mg/dl, dilute the sample and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

8.1 – 10.4 mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

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Limitations

- The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.
- Calcium contamination should be avoided. It is recommended to use unused plastic tubes or cuvettes free from calcium. For glassware use the washing solution to avoid contamination.
- Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample balnk may be used as mentioned below:
 - Add 10µl of serum sample to 1000µl of DI water and read absorbance at 650 nm.
 - Subtract the absorbance obtained as above, from the absorbance of test. Use this corrected absorbance for calculation.
 - SI conversion factor: 1mg/dl = 1mmol/l x 4

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for calcium.

Performance

Linearity Limit: 15mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	9.92	12.50
S.D.	0.14	0.33
C.V. %	1.40	2.61

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	9.72	12.42
S.D.	0.14	0.23
C.V. %	1.43	1.80

References

- Marshall, W. J. 1995. Clinical Chemistry, 3rd ed. Mosby, London.
- Tierney, Lawrence M.; McPhee, Stephen J.; Papadakis, Maxine A. (2006). Current Medical Diagnosis and Treatment 2007 (Current Medical Diagnosis and Treatment). McGraw-Hill Professional. pp. 901. ISBN 0-07-147247-9.
- Tietz N.W., (Ed.), Textbook of Clinical Chemistry, W.B. Saunders, (1986), p.1350.
- Baurer P.J. Anal. Biochem 1981: 110:61-72.
- Farrell C.E. "Electrolytes" in Clinical Chemistry Theory. Analysis and correlation. The C.V. Mosby Company. Kaplan L.A., Pesce A.J. (Ed). 1984; Chap 55; 1054

Pack Presentation

Product Code/ Catalogue No.	KGCAR105.2.1
Pack Size*	2X50ml
Reagent 1	2X50ml
Reagent 2 (Washing soln.)	1X50ml
Reagent 3	1X2ml










* Pack size may vary on market demand.

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Symbols

Following symbols are used in the labeling of KeeGad kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions Storage temperature
	In Vitro Diagnostics		
	Expiry Date		Content
	Product Name		Manufacture



Manufactured by:
KEE GAD Biogen Pvt. Ltd.
A-8, Third Floor, Naraina Industrial Area,
Phase-II, New Delhi-110028 (India)