

**Intended Use**

For **IN VITRO** quantitative determination of Calcium in serum using manual or automated applications.

**Clinical Significance**

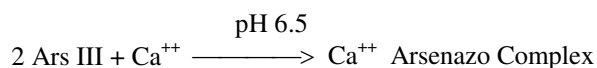
Measurements of Calcium activity are primarily used for diagnosing hyperparathyroidism, steatorrhea, nephrosis, nephritis and pancreatitis, as well as for monitoring the causes and treatments. (1)

**Method History**

In 1971 Michaylova and Ilkova (2) reported the use of Arsenazo-III (o-(1, 8 dihydroxy-3, 6-disulfonaphthylene-2, 7 bisazo)-bis benzene-arsonic acid) for the quantitative determination of micro amounts of Calcium in the presence of other metallic ions. The authors found that under acidic pH, magnesium does not interfere with the  $\text{Ca}^{++}$  reaction since at this pH Arsenazo-III does not form complexes with  $\text{Mg}^{++}$ . The strong specific affinity and its micromolar dissociation constant for the dye-calcium complex makes this metallochromic indicator Arsenazo-III a most suitable reagent for an accurate and rapid determination of  $\text{Ca}^{++}$  in serum samples.

**Method Principle**

The serum sample is mixed with the Calcium Working Reagent. The total free Calcium reacts at 25 degrees C with Arsenazo-III at pH 6.5 to form a purple-colored complex with maximum absorbance at 650nm. The increase of the purple-colored complex is directly proportional to the concentration of Calcium in the sample. The reaction scheme illustrates the reaction that occurs in the method.

**Reagent Content**

When reconstituted according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows.

**Calcium Arsenazo III Reagent**

Each liter contains:

Buffer

Arsenazo-III 0.15 mmol/L

Nonreactive ingredients and stabilizer

**Preparation Of Working Reagent**

Catachem Calcium Arsenazo-III Color Reagent is packaged in ready-to-use form. No preparation is required.

**Reagent Storage And Stability**

Store Catachem Calcium Arsenazo-III Reagent at room temperature. When stored as directed, the reagent is stable until the expiration date stated on the label.

**Specimen Collection And Preparation**

Test specimens should be fresh, clear unhemolyzed sera. Old serum samples are not recommended since with age, protein will denature and precipitate together with calcium. Blood collected with anticoagulants such as oxalates and EDTA should be avoided since these anticoagulants will either precipitate or chelate  $\text{Ca}^{++}$  ions thus producing erroneous results.

**Precautions**

Avoid contact of specimens with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

**Quality Control**

To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of a normal and abnormal control serum.

**Interfering Substances**

Several substances have been reported to interfere with the Calcium method. (5) Care must be taken to use clean glassware and calcium-free distilled or deionized water. Anticoagulants such as oxalates and fluorides will depress calcium values. A summary of interference of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (5)

**Expected Values**

The range of expected values determined for this method is 8.5 mg/dL to 10.5 mg/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located. (1)

**Directions For Use**

Catachem's Calcium Arsenazo III method requires a single reagent. The reagent is packaged ready for use. No preparation is required. The Working Reagent is stable for twelve months aboard the analyzer at 2-8°C if capped while not in use.

## Procedure

**Important:** Read entire procedure instructions before proceeding with assay.

## Materials Required (Not Provided)

Spectrophotometer  
Match cuvettes 1 cm light path  
Timer to time incubation time  
Pipette 1.0 ml for reagent  
Pipette 0.02 ml for sample

## Materials Provided

Catachem Calcium Reagent

## Analytical Parameters

Wavelength 650 nm or (600nm)  
Pathlength 1 cm  
Reaction Mode endpoint  
Reaction Time 2.0 minute  
Reagent Volume 1.0 ml  
Sample Volume 0.02  
Total Volume 1:02 ml  
Sample-to-reagent ratio 1:51

## Assay Procedures

1. Pipette 1.0 ml of Calcium Reagent into each of three cuvettes marked "Calibrator", "Sample" and "Blank".
2. Pipette 0.02 ml of calibrator or sample into their respective cuvettes. Use 0.02 ml of distilled water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for 2.0 minute at room temperature.
4. Set spectrophotometer wavelength at 650 nm (or 600 nm) and zero the instrument with the blank.
5. Read the "Calibrator" and "Sample" absorbencies.
6. Calculate the Calcium concentration (mg/dL) in the sample(s), as shown in calculations and results.

## Calculations and Results

$$\text{Calcium (mg/dL)} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (mg/dL)}$$

Example:            Assay OD  
Sample            0.240  
Calibrator        0.250

$$\text{Calibrator} = 10.0 \text{ mg/dL}$$

$$\text{Calcium (mg/dL)} = \frac{0.240}{0.250} \times 10.0 \text{ mg/dL}$$

$$= 9.6 \text{ mg/dL}$$

## Method Performance Characteristics

**Sensitivity:** The sensitivity of this method under normal circumstances is to 0.1 mg/dL (0.025 mmol/L)

**Linear Range:** In this method there is no significant nonlinearity over the range of 2.0 - 20 mg/dL. (0.5 - 5 mmol/L)

**Precision:** Within-run and day-to-day precision is summarized in the following table:

## Precision Study

Calcium Mean mg/dL	Within-Run Precision		Total Precision	
	SD mg/dL	CV %	SD mg/dL	CV %
2.6	0.00	0.00	0.22	8.40
12.3	0.00	0.00	0.18	1.40
20.4	0.13	0.64	0.36	1.80

## Correlation

A comparison of this method using an automated analyzer and a reference method based upon the Arsenazo-III reaction resulted in the following regression statistics.

$$\begin{aligned} \text{Range} &= 7.5 - 15.3 \text{ mg/dL} \\ N &= 106 \\ Y &= 0.965x + 0.35 \\ r &= 0.992 \\ S_{y.x} &= 0.17 \end{aligned}$$

## References

1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Michaylova V and Ilkova P. Anal Chim Acta 53, 194-198, 1971.
3. Bauer PJ. Anal Biochem 110, 61-72, 1981.
4. Ogan K. Simons ER. Anal Biochem 96, 70-76, 1979.
5. Young DA, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21 (5) (1975).

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