# CALCIUM (ARSENAZO III)

**INTENDED USE**
Bioline CALCIUM ARSENAZO III reagent is for the direct, colorimetric determination of calcium in human serum or urine.

**CLINICAL SIGNIFICANCE**
More than 99% of body calcium exists in bones and teeth. The remaining 1% is present in blood and soft tissues and serves as a cofactor in blood coagulation, metabolism, and neuromuscular physiology. Serum calcium is present in three different forms: 1) nearly 45% is bound by serum proteins, 2) about 5% is complexed in a non-ionized form and 3) the remaining 50% serum calcium is in an ionic (free) form. It is the physiologically active ionic fraction that is important in terms of biological function.
Many factors influence serum calcium levels: hypercalcemia (increased serum calcium) is observed in hyperparathyroidism, hypervitaminosis, sarcoidosis, myeloma, and certain cancers of the bone. Hypocalcemia (decreased serum calcium) is encountered in hypoparathyroidism, rickets, nephrosis, nephritis, steatorrhea, and pancreatitis. Any decrease in serum proteins frequently results in a decrease of the total serum calcium level. Similarly, an increase in protein such as in myeloma may increase the total serum calcium level. There also appears to be a reciprocal relationship between calcium and phosphorus. Increases in serum inorganic phosphorus are associated with a decrease in serum calcium.

**METHOD AND PRINCIPLE**
Earlier procedures for the determination of calcium involved precipitation of calcium and subsequent determination of the anion of the precipitating agent. More recently, calcium compounds have been determined by atomic absorption spectrophotometry, which has subsequently been recommended as the reference method for determining total serum calcium. Atomic absorption spectrophotometry involves the use of an expensive and dedicated instrument. With the development of chelating reagents and metallochromic indicators, the atomic absorption methods were rapidly replaced by complex metric procedures, which can measure calcium in the serum directly.

The method is based on the specific binding of Arsenazo III and calcium at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of total calcium in the sample.

**REAGENT COMPOSITION**
***Reagent:***

| | |
|---|---|
| MES Buffer, pH 6.50 | 100 mmol/L |
| Arsenazo III | 200 µmol/L |
| ***Standard:*** | 10 mg/dL |

**WARNINGS AND PRECAUTIONS**
1. For *in vitro* diagnostic use.
2. *CAUTION: In vitro* diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures, which dictate avoiding ingestion and eye or skin contact.

**REAGENT PREPARATION**
Reagent and Standard are ready to use..

**REAGENT STORAGE AND STABILITY**
- All reagents should be stored at room temperature (15 - 30°C).

**REAGENT DETERIORATION**
The reagent should be discarded if:
- Turbidity has occurred; turbidity may be a sign of contamination.
- The reagent fails to meet linearity claims or fails to recover control values in the stated range.

**SPECIMEN COLLECTION AND STABILITY**
Serum***:***
- Fasting non-hemolyzed serum is specimen of choice.
- Anti coagulants other than heparin should not be used.
- Remove serum from clot as soon as possible since red cells can absorb calcium.
- Older serum specimens containing visible precipitate should not be used.
- Tubes with cork stoppers should not be used.
- Serum calcium is stable for 24 hours at room temperature (15 - 30°C), 1 week refrigerated (2 - 8°C) and up to 5 months frozen and protected from evaporation.

Urine:
- Collect 24 hours urine in a dry clean container containing 20-30 ml of 6N HCl.
- Urine diluted 1/3 with distilled water. Take dilution factor into account for the calculation of the concentration in urine.

**INTERFERENCES**
- Substances that contain calcium or complex calcium should not come in contact with the test specimen. Examples: EDTA, citrate, oxalate, and fluoride.
- Specimens from patients receiving bromsulfophthalein (BSP) or EDTA should not be used.
- For a list of substances affecting the accuracy of calcium values with this procedure refer to the references.

**ASSAY PROCEDURE FOR SEMIAUTO ANALYZER**
Wavelength : 650 (630 - 670)nm
Temperature : RT

| | Blank | Standard | Sample |
|---|---|---|---|
| **Reagent** | 1000 µL | 1000 µL | 1000 µL |
| **Standard** | - | 20 µL | - |
| **Sample** | - | - | 20 µL |

Mix and read the optical density (OD) of standard and sample against reagent blank after 1 minutes of incubation at RT. The final colour is stable for at least next 1 hour.

**CALCULATION**
**Abs. of Unknown x Conc. of std. = Calcium (mg/dl)**
**Abs of Standard**
*Example:* If the Absorbance of unknown = 0.74,
Absorbance of standard = 0.84,
Concentration of standard = 10 mg/dl,
then, calcium mg/dl
Calcium mg/dl = $\frac{0.74 \times 10}{0.84}$ = 8.8 mg/dl

**Urine**
$\frac{\text{A Sample}}{\text{A Standard}} \times \text{Standard Concn} \times F = \text{mg/24-hours total calcium}$
F = Dilution factor = 2

**CALIBRATION**
The procedures are calibrated with the standard solution which is included with each series of tests. Its absorbance is used to calculate results. It is recommended to establish a linearity curve up to 20 mg/dl with other available commercial standard solutions to verify the performance of the instruments and reagents.

**LIMITATIONS**

The reagent is linear to 20 mg/dl. Samples with values above 15 mg/dl should be diluted 1:1 with saline, re-assayed and the result multiplied by 2.
Lipemic or hemolyzed samples require a serum blank.
To prepare a serum blank add 0.02 ml (20 µl) of sample to 1.0 ml distilled water.
Mix and read against water at 650 nm (630 - 670 nm) nm. Subtract the absorbance reading from the test reading and perform calculation

**QUALITY CONTROL**

To ensure adequate quality, control sera normal and abnormal control should be used. These controls must be performed & validated before the patient samples are assayed. The control frequency must be at least once a day, after each calibration and should be adapted to Quality Control procedures of each laboratory and the regulatory requirements. Results should be within the defined ranges. If values fall outside of the defined ranges, each laboratory should take corrective measures. Quality control material should be used in accordance with local guidelines.

**EXPECTED VALUES**

SERUM : 8.5 – 10.5 mg/dl

**URINE REFRENCE RANGE**

Adults (normal diet)
100-300 mg/24-h.

**PERFORMANCE CHARACTERISTICS**

**- *Precision***

Determined according to CLSI EP5-A2 protocol

**- Correlation**

Serum/Plasma

A comparative study has been performed between Bioline Calcium and commercially available Calcium Arsenazo III reagent using human sera samples according to CLSI EP9-A2 protocol.
The sample concentrations were between 5.07 and 14.79 mg/dL.
The parameters of linear regression are as follows:
Correlation coefficient: (r) = 0.993
Linear regression: y = 0.949 x + 0.41 mg/dL.

**General Technical Parameters**

| | |
|---|---|
| **Mode** | **End Point** |
| **Wavelength (Filter)** | **650 (630 – 670) nm.** |
| **Reaction Direction** | **Increasing** |
| **Reagent Blank** | **Yes** |
| **Sample Vol.** | **20 µL** |
| **Reagent Vol.** | **1000 µL** |
| **Incubation Time** | **1 min at RT** |
| **Reagent Blank Abs (Max.)** | **NMT 1.000 abs @650nm** |
| **Calibration Method** | **1 - Point** |
| **Standard (Conc.)** | **10 mg/dL** |
| **Linearity** | **20 mg/dL** |
| **Decimal Places** | **2** |
| **Temp.** | **RT** |
| **Unit** | **mg/dL** |
| **Ref. Low (Male / Female)** | **8.5 mg/dL** |
| **Ref. High (Male / Female)** | **10.5 mg/dL** |

**REFERENCES**

Young, D.S., Effects of preanalytical variables on clinical laboratory tests, 2nd edition, AACC Press (1997).
Young D.S., Effects of drugs on clinical laboratory tests, 4th edition, AACC Press (1995).