

Calcium (AZIII)

1.0 INTENDED USE

This reagent is intended for the quantitative determination of total calcium concentration in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Historically, serum calcium has been determined by a variety of procedures including flame photometry, atomic absorption spectrophotometry and recently specific dye binding such as o-cresolphthalein complexone. Accurate and precise measurement of calcium in biological fluids has traditionally been difficult (10.1.) The introduction of Arsenazo III by Michaylova and Illkova (10.3) provided a highly sensitive and very specific reagent for calcium determination. The King method is based on this dye-binding method.

2.2 TEST PRINCIPLE

This method for the determination of calcium presented here uses Arsenazo III (3,6-bis ((2-Arsonophenyl) Azo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid) (10.2); CAS registry number: 1668-00-4. Arsenazo III is chemically stable and has a very high affinity for calcium in a neutral pH range. In this assay system, the Arsenazo III forms a 1:1 violet Arsenazo III: calcium complex with an absorbance maximum at 650nm (10.3.) The concentration of calcium is proportional to the absorbance of the violet colored Arsenazo III: calcium complex. The color produced by this complex is stable for at least 8 hours at room temperature (18-26°C).

2.3 CLINICAL SIGNIFICANCE (10.8)

The normal calcium concentration of serum is maintained by hormones in the parathyroid gland. Decreased levels occur in hypoparathyroidism, vitamin D deficiency, rickets, osteomalacia and renal tubular acidosis. Increased levels are found in hyperparathyroidism, vitamin D intoxication, and are associated with neoplasms, especially those of bone.

A significant fraction of serum calcium is bound to protein. Hyperproteinemia is associated with an increased level of serum calcium and hypoproteinemia is associated with a decreased level of serum calcium.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Heparinized plasma may also be used. Plasma prepared using EDTA, oxalate, citrate, which function by removal of calcium, obviously must not be used.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 µl. Call Biotron's technical service department at 1-800-595 -8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, the serum sample can be stored refrigerated (2-8°C) for up to 7 days.

4.1 MATERIALS

(1 X 500 ml)

Reagents necessary for the determination of calcium are included in the kit.

4.1 REAGENT

Calcium reagent contains:

Arsenazo III 290 mmol/L
buffer solution 75 mmol/L
preservative and a stabilizer

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.2.1 DO NOT pipet by mouth. DO NOT ingest.

4.2.2 Organic arsenic compounds have been classified as potentially carcinogenic; therefore, safe laboratory practices should be carefully observed.

4.2.3 Components of the reagent may be irritating to the skin and mucous membranes; avoid contact. If contact occurs, wash with copious amounts of water.

4.3 REAGENT PREPARATION

The reagent is ready to use as is.

If the absorbance of the reagent alone (without sample added) in a 1 cm cuvette is greater than 0.500 when measured against distilled water at 650 nm, do not use the reagent.

4.4 REAGENT STORAGE AND STABILITY

The reagent is stable at room temperature (18-26°C) until the expiration date on the label.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 650 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 650 nm.

4.5.3 Test tubes capable of holding 2 ml.

4.5.4 Pipettes capable of delivering 1 ml and 25 µl.

4.5.5 Timer for 1 minute incubation.

4.5.6 Distilled or deionized water.

4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	650 nm
Temperature	18-26° C or 37° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	1 minute
Sample volume	25 µl
Reagent volume	1 ml
Total volume	1.025 ml
Sample to reagent ratio	1/40

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 650 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The calcium assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

5.4.1 Prepare the required volume of calcium reagent.

5.4.2 Into separate calcium free test tubes pipette 25 µl of distilled water, calibrator or serum to be assayed.

5.4.3 Add 1 ml of calcium reagent and mix.

5.4.4 Incubate for 1 minute at room temperature and determine the absorbance of the calibrator (As) and of each serum (A) at 650 nm using the distilled water sample as the reagent blank.

5.5 PROCEDURE NOTE

The color is stable for 8 hours.

A major source of difficulty with the assay of calcium is contaminated glassware employed in the performance of the test. Many detergents and water supplies contain calcium and incompletely rinsed containers used in this test will lead to inaccurate results.

5.6 CALCULATION AND RESULTS

$$\text{Calcium} = \frac{A}{A_s} \times \text{concentration of calibrator}$$

where A = absorbance of sample, A_s = absorbance of calibrator.

$$\text{Example: Calcium} = \frac{0.581}{0.645} \times 10 \text{ mg/dl} = 9.0 \text{ mg/dl}$$

where A = 0.581, A_s = 0.645, concentration of calibrator = 10 mg/dl.

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.5,10.6)

The range of expected values is: 8.5 to 10.5 mg/dl (2.1 to 2.6 mmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.9)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

Any substance which either chelates calcium or contains calcium will interfere with the assay.

Magnesium does not interfere in this assay system. The affinity of Arsenazo III for magnesium is essentially zero at the pH at which the assay is performed (10.2.)

When magnesium concentrations in incremental amounts of 1,2,4 and 6 mg/dL (magnesium expected range value: 1.8 to 2.9 mg/dL) were added to 12 serum samples with calcium values ranging from 7.2 to 9.8mg/dL, there was no increase in the calcium values. There was also no increase due to magnesium in the calcium value (9.8 ± 0.9 mg/dl) in 12 assays of a control serum to which magnesium was added in concentrations ranging from 0.5 to 15mg/dL (10.4.)

Young et al. (10.4) have published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that for serum calcium.

Interference from lipemia is minimized because of the small amount of sample used.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The calcium assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 10 replicates of 3 control sera were run.

	<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
Serum 1	6.2	± 0.09	1.5
Serum 2	15.2	± 0.15	.98
Serum 3	21.0	± 0.34	1.6

Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 3 control sera.

	<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
Serum 1	6.1	± .12	1.9
Serum 2	15.2	± .22	1.5
Serum 3	20.6	± .29	1.4

9.2 CORRELATION

A correlation study was done comparing this method (y) with a calcium o-cresolphthalein method as the reference method (x₁). 76 samples were run with a range between 6.2 mg/dl to 18.6 mg/dl. A second correlation study was done comparing this method (y) with an atomic absorption method as the reference method (x₂). 20 samples were run with a range between 6.2 mg/dl to 19 mg/dl. The results are summarized below.

<u>Number of Samples</u>	<u>Regression Equation</u> y=Biotron, x=Comparative	<u>Correlation Coefficient</u>
76	y = .993 x ₁ + .060	0.994
20	y = .960 x ₂ + .364	0.997

9.3 LINEARITY

The assay is linear to 15 mg/dl calcium. Samples with calcium concentrations exceeding 15 mg/dl should be diluted with an equal volume of distilled or deionized water and the assay repeated. Multiply results by 2.

9.4 SENSITIVITY

Using a 1:40 sample to reagent ratio and reading at 650nm, a 1 mg/dl calcium sample will produce a net absorbance of approximately 0.051.

10.0 REFERENCES

- 10.1 Textbook of Clinical Chemistry, Edited by N.W. Tietz, p. 1342. W.B. Saunders Company, Philadelphia, 1986.
- 10.2 Bauer, P.J. Anal. Biochem. 110, 61-72, 1981.
- 10.3 Michaylova, V., and Illkova, P., Anal Chim Acta 53, 194-198, 1971.
- 10.4 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
- 10.5 Todd, Sanford and Davidsohn, Clinical Diagnosis and Management by Laboratory Methods, Edited by Henry, J.B., W.B. Saunders Company, Philadelphia, 1979.
- 10.6 Tietz, N.W., Clinical Guide to Laboratory Tests, p. 92, W.B. Saunders Company, Philadelphia, 1983.
- 10.7 Hoffman, W.S., The Biochemistry of Clinical Medicine, 4th Ed., 548-613, Year Book Medical Publishers, Inc., Chicago, 1970.
- 10.8 Todd, Sanford and Davidsohn, Clinical Diagnosis and Management by Laboratory Methods, Edited by Henry, J.B., W.B. Saunders Company, Philadelphia, 1969, p.575.
- 10.9 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704