

Calcium Reagent

Catalog #: 43709

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Calcium in serum.

CLINICAL SIGNIFICANCE^{1,2}

Increased serum calcium may be observed in hyperparathyroidism, vitamin D intoxication, multiple myeloma and some neoplastic diseases of bone. Decreased serum calcium may be observed in hypoparathyroidism, vitamin D deficiency, steatorrhea, nephrosis, and nephritis.

METHODOLOGY

Various methodologies have been developed for the determination of calcium including flame photometry, fluorescent, gravimetric and titrimetric procedures, ion selective electrodes, and atomic absorption. Atomic absorption has been recommended as the reference method but it requires expensive instrumentation.³

Specific dye binding methodologies have become popular for calcium determination because they are rapid, convenient and inexpensive. Procedures using the dyes alizarin-3-sulfonate and methylthymol blue have been described.^{4,5} A method using o-Cresolphthalein Complexone as the chromagen was developed in 1966 by Connerty and Biggs, and modified by Gitelman in 1967 and Baginski, et al in 1973.^{6,7,8} O-Cresolphthalein Complexone procedures have been widely used for the determination of calcium.

The present procedure uses Arsenazo III and has been modified to provide a highly sensitive and stable reagent system. The reagent is provided as a convenient ready to use liquid.

Principle



Calcium reacts with Arsenazo III in a slightly alkaline medium to form a purple-colored complex which absorbs at 650 nm. The intensity of the color is proportional to the calcium concentration.

REAGENT COMPOSITION

Active Ingredients	Concentration
Arsenazo III	0.2 mM
Imidazol Buffer	100 mM
Surfactant.	
pH 6.75 ± 0.2	

PRECAUTIONS

1. Reagent is for *in vitro* diagnostic use only.
2. Reagent may be irritating to the skin. Avoid contact. Flush with water if contact occurs.
2. Reagent contains Sodium Azide as a preservative. In a dried form this may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

REAGENT PREPARATION

Reagent is supplied ready to use.

STABILITY AND STORAGE

When stored at 2-25°C, the reagent is stable until the expiration date stated on the label.

REAGENT DETERIORATION

The reagent should not be used if:

1. The reagent is turbid.
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND STORAGE

1. Fresh, unhemolyzed serum is the preferred specimen.
2. Anticoagulants other than heparin should not be used.⁹
3. Remove serum from clot as soon as possible since red cells can absorb calcium.¹⁰
4. Older serum specimens containing visible precipitate should not be used.^{11,12}

5. Serum calcium is stable for one week at refrigerated (2-6°C), and up to five months frozen (-15 to -25 °C) when protected from evaporation.¹³ Specimens should not be thawed and then refrozen.

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (<5%) from hemoglobin up to 600 mg/dL.

Bilirubin:

No significant interference (<5%) from bilirubin up to 15 mg/dL.

Lipemia:

No significant interference from lipemia (<5%) up to 278 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.¹⁴

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. SDI CA480 analyzer.
2. Deionized water and related equipment, e.g.: pipettes
2. Analyzer specific consumables, e.g.: sample cups
3. Control, and Calibrator materials obtained from SDI Biomed.

ASSAY PROCEDURE

System Parameters

Calcium TEMPERATURE:	37°C
WAVELENGTH:	650 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 50
e.g. Sample Vol.	0.02 mL (20mL)
Reagent Vol.	1.0 mL
INCUBATION;	3 1 min

Procedure Notes

1. Final color is stable for 60 minutes.
2. Samples with calcium above 15 mg/dL should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
3. Severely lipemic samples may require a serum blank.
4. Contamination of glassware with calcium will adversely affect test results. Acid-washed glass or plastic test tubes are recommended.

Calculation

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

Example:

Absorbance of sample = 0.81
Absorbance of standard = 0.80
Concentration of standard = 10 mg/dL

$$\frac{0.81}{0.80} \times 10 = 10.1 \text{ mg/dL Calcium}$$

LIMITATIONS

1. Samples with calcium values exceeding 15mg/dL should be diluted with an equal volume of distilled water, the assay repeated, and the result multiplied by two.
2. Severely lipemic samples should be run with a serum blank for greatest accuracy.

CALIBRATION

Use an aqueous Calcium standard or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reagent should be monitored by use of a two level control with known Calcium values.

EXPECTED VALUES

Adults: 8.5 - 10.4 mg/dL¹⁵
Newborns: 7.8 - 11.2 mg/dL¹⁶

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity

When run as recommended the assay is linear from 0.1 to 15.0 mg/dL.

Method Comparison

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	46
Range of samples:	5.1 – 13.0 (mg/dL)
Correlation Coefficient:	0.9924
Slope:	1.022
Intercept:	-0.44 (mg/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (mg/dL)	5.24	10.34	12.16
S.D. (mg/dL)	0.07	0.11	0.13
C.V. (%)	1.2	1.1	.1

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)			
Mean (mg/dL)	5.24	10.34	12.16
S.D. (mg/dL)	0.09	0.19	0.24
C.V. (%)	1.8	1.8	2.0

Sensitivity

A calibration factor of approximately 31.8 was obtained, which is equivalent to a sensitivity of 0.031 D Abs per mg/dL.

REFERENCES

1. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.149 (1984).
2. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods, Philadelphia, W.B. Saunders, p.149 (1984).
3. Call, J.P., et al, N.B.S., Sp. Publication 260:36 (1972).
4. Connerty, H.V. and Biggs, A.R., Clin. Chem. 11:716 (1965).
5. Gindler, E.M. and King, J.D., Am. J. Clin. Path. 58:376 (1972).
6. Connerty, H.V. and Biggs, A.R., Am. J. Clin. Path. 45:290 (1966).
7. Gitelman, H.J., Anal. Biochem. 18:521 (1967).
8. Baginski, E.S., St et al, Clin. Chem. Acta 46:49 (1973).
9. Richterich, R., Clinical Chemistry: Theory and Practice, New York, Academic Press, p.304 (1969).
10. Peters, J.P., Van Slyke, D.D., Quantitative Clinical Chemistry - Vol 2, Baltimore, Williams and Wilkins, (1932).
11. Chen, P.S., et al, Anal. Chem. 26:1967 (1954).
12. Teyeau, F., et al, Bull. Soc. Pharm. Bordeaux, 95:206 (1956).
13. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, Hagerstown (MD), Harper and Row, p. 669 (1974).
14. Young, D.S., et al, Clin. Chem. 21:1D (1975).
15. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, WE. Saunders, p. 1208 (1984).
16. Meites, Samuel, Pediatric Clinical Chemistry, Washington DC, AACC Press, p. 81 (1989).

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