

CONCEPT DIAGNOSTICS

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CALCIUM REAGENT SET

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For the direct, colorimetric determination of calcium in serum or urine.

INTRODUCTION

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 nonionized 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.¹

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.² 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 complexometric procedures which can measure calcium in the serum directly.^{3,4,5}

PRINCIPLE

Calcium + O-Cresolphthalein Complexone $\xrightarrow[\text{Medium}]{\text{Alkaline}}$

Calcium - Cresolphthalein Complexone Complex (purple color)

Calcium reacts with cresolphthalein complexone in 8-hydroxyquinoline to form a colored complex (purple color) that absorbs at 570 nm (550 - 580). The intensity of the color is proportional to the calcium concentration. Color intensifiers and a stabilizer are present to minimize interference by other metallic ions.

REAGENT COMPOSITION

When reconstituted as directed, the reagent for calcium contains the following:

1. Calcium Color Reagent (A): O-Cresolphthalein Complexone 0.14 mM, 8-Hydroxyquinoline 13 mM
2. Calcium Buffer: Diethylamide 363 mM, Potassium Cyanide, 2 mM, Nonreactive ingredients and stabilizers in both reagent A and B.

3. Calcium Standard.: Calcium Carbonate in dilute hydrochloric acid. (10 mg/dl).

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion and eye or skin contact.
2. Reagent (A) and (B) may be irritating to skin. Avoid contact..
3. Reagent (B) contains a cyanide and should **NOT BE PIPETTED BY MOUTH.**

REAGENT PREPARATION

1. Combine equal volumes of Calcium Color Reagent (A) and Calcium Buffer (B), mix and let stand for ten (10) minutes at room temperature before use.
2. Reagents should be combined in clean plastic vessels. Water and Glassware containing calcium will react with the reagent. All glassware should be rinsed in diluted hydrochloric acid before use.

STORAGE AND STABILITY

1. All reagents should be stored refrigerated (2 - 8°C).
2. Combined reagent (A and B) is stable for two (2) weeks refrigerated and one (1) week at room temperature. Keep bottles tightly capped to prevent evaporation.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN COLLECTION

serum:

1. Fasting nonhemolyzed serum is specimen of choice.
2. Anticoagulants other than Heparin should not be used.⁶
3. Remove serum from clot as soon as possible since red cells can absorb calcium.⁷
4. Older serum specimens containing visible precipitate should not be used.^{8,9}
5. Tubes with cork stoppers should not be used.¹⁰
6. Serum calcium is stable for twenty-four (24) hours at room temperature, one (1) week refrigerated (2 - 8°C) and up to five (5) months frozen and protected from evaporation.¹¹

urine:

1. Collect 24 hours urine in a dry clean container containing 20-30 ml of 6N HCl.
2. Alternatively use 1-2 ml of 6N HCl for random sample.

INTERFERING SUBSTANCES

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices.
2. Test tubes/rack.
3. Timer.
4. Spectrophotometer able to read at 570 nm.

GENERAL INSTRUCTIONS

The reagent for Calcium is intended for use as either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (MANUAL)

1. Prepare working reagent. See "REAGENT PREPARATION."
2. Label tubes Blank, Standard, Controls, Patients, etc.
3. Transfer 1.0 ml of working reagent into each tube.
4. Add 0.02 ml (20 μ l) of sample to respective tubes and mix.*
5. Let stand for at least sixty (60) seconds at room temperature.
6. Zero spectrophotometer with blank at 570 nm.(Wavelength range:500-500).
7. Read and record absorbances of all tubes. Final color is stable for twenty (20) minutes.

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

*ALTERNATIVE VOLUMES: (0.05 ml sample to 3.0 ml reagent).

PROCEDURAL LIMITATIONS

The reagent is linear to 20 mg/dl.

1. Samples with values above 20 mg/dl should be diluted 1:1 with saline, re-assayed and the result multiplied by two (2).
2. Lipemic or hemolyzed samples require a serum blank. To prepare a serum blank add 0.05 ml (50 μ l) of sample to 3.0 ml distilled water. Mix and read against water at 570 nm. Subtract the absorbance reading from the test reading and perform calculation.
3. Contamination of glassware with calcium (usually from detergents) will adversely affect the test. Use acid-washed glassware of plastic tubes.

CALCULATIONS

$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of std.} = \text{Calcium (mg/dl)}$

Example: If the absorbance of unknown = 0.74. absorbance of standard = 0.84, concentration of standard = 10 mg/dl, then,

$$\frac{0.74}{0.84} \times 10 = 8.8 \text{ mg/dl}$$

Note: To convert mg/dl to meq/L, divide mg/dl by two (2).

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established calcium values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES¹²

8.5 - 10.5 mg/dl.

Children under 12, usually have high normal values which decrease with aging.

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

PERFORMANCE CHARACTERISTICS

1. Linearity: 20 mg/dl.
2. Comparison: A study performed with a similar method yield a correlation coefficient of 0.97 with a regression equation of $y = 0.94x + 0.53$.
3. Precision studies:

	Within Run	
<u>Mean (mg/dl)</u>	<u>SD</u>	<u>C.V.</u>
9.1	0.39	4.3%
13.7	0.02	0.2%

	Run to Run	
<u>Mean (mg/dl)</u>	<u>SD</u>	<u>C.V.</u>
9.2	0.21	2.2%
13.3	0.32	2.4%

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