TECO DIAGNOSTICS
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CALCIUM REAGENT SET

INTENDED USE
For the direct, colorimetric determination of calcium in human 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 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.[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 complex metric 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 nm). 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, Non-reactive ingredients, and stabilizers in both reagents 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 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.

REAGENT STORAGE AND STABILITY
1. All reagents should be stored at room temperature (15 - 30°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 non-hemolyzed serum is specimen of choice.
2. Anti coagulants 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 (15 - 30°C), 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 bromsulfophthalein (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.

MANUAL PROCEDURE

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 µl) of sample to the respective tubes and mix*.
5. Let stand for at least sixty seconds (60) at room temperature.
6. Zero spectrophotometer with blank at 570 nm. (Wavelength range: 550 - 600 nm)
7. Read and record absorbances of all tubes. Final color is stable for twenty minutes (20).

** TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.*

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

LIMITATIONS

1. The reagent is linear to 20 mg/dl. 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 µl) 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 or 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 reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

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 range of expected values, since differences exist between instruments, laboratories, and local populations.

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 $y = 0.94 x + 0.53$.
3. *Precision:*

	Within Run	
Mean (mg./dl)	S.D.	C.V. (%)
9.1	0.39	4.3%
13.7	0.02	0.2%

	Run to Run	
Mean (mg./dl)	S.D.	C.V. (%)
9.2	0.21	2.2%
13.3	0.32	2.4%

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