

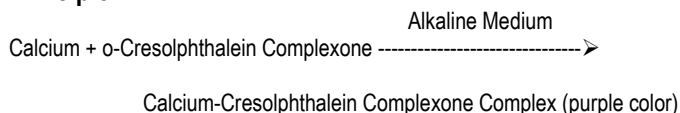
Intended Use

For the quantitative determination of Calcium in serum.

Method History

The various methods developed for the determination of calcium include colorimetric, fluorescent, gravimetric, ion selective, titrimetric, and atomic absorption. The atomic absorption method has been recommended as the reference method but it requires complicated and expensive equipment.¹ A rapid, convenient, simple and inexpensive method is one based on the interaction of calcium with a suitable chromogenic agent. o-Cresolphthalein Complexone was first used as a complexing reagent for metals in 1954.^{2,3} It was adapted to a spectrophotometric method for determining calcium in 1956.⁴ Gitelman modified the procedure in 1967 to include 8-hydroxyquinoline to remove magnesium interference and used diethylamine for a buffer pH of 12.⁵ Baginski, et al modified the procedure further in 1973 to include DMSO to lower the dielectric constant of the working reagent and repress ionization of the o-Cresolphthalein Complexone.⁶ The present procedure is based on modifications of the above methods.

Principle



Calcium reacts with CPC in an alkaline medium to form a purple-color that absorbs at 570nm (550-580nm). The intensity of the color is proportional to the calcium concentration.

Reagents

1. Color Reagent: o-Cresolphthalein Complexone 0.11mM, 8-Hydroxyquinoline 17.0mM, surfactant.
2. Buffer Reagent: 2-Amino-2-Methyl-1-Propanol 976mM, 2.0mM Potassium Cyanide

Reagent Preparation

1. Combine equal volumes of color and buffer reagent, mix and let stand for twenty 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

1. All reagents should be stored refrigerated (2-8°C).
2. Combined reagent is stable for two weeks refrigerated and one week at room temperature. Keep bottles tightly capped to prevent evaporation.
3. The sensitivity of the working reagent will decline with time. A standard must be included in each run.
4. Labs using profiling instruments, that run a standard at the beginning of the day and use the factor from that run to standardize subsequent runs, must store the working reagent at 2-8°C and use it cold. This is done to lessen sensitivity drift.

Reagent Deterioration

The reagent should be clear; turbidity indicates deterioration and should not be used.

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Buffer Reagent contains a POISON and should NOT BE PIPETTED BY MOUTH.
3. Color and Buffer Reagent may be irritating to skin. Avoid contact.

Specimen Collection and Storage

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

Interferences

1. Substances that contain calcium or complex calcium should not come in contact with the test specimen. Examples: EDTA, citrate, oxalate, fluoride.
2. Specimens from patients receiving bromosulphthalein (BSP) or EDTA should not be used.¹³
3. For a list of substances affecting the accuracy of calcium values with this procedure see references 14 and 15.

Materials Provided

Calcium Reagent Set.

Materials Required but not Provided

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

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Prepare reagent. See "Reagent Preparation".
2. Label test tubes "blank", "standard", "control", "sample", etc.
3. Pipette 1.0 ml of reagent into each tubes.*
4. Add 0.02ml (20ul) of sample to respective tubes and mix.
5. Let stand for at least one minute at room temperature.
6. Zero spectrophotometer with blank at 570nm.
7. Read and record absorbances of all tubes. Final color is stable for twenty minutes.

* For spectrophotometers requiring greater than 1.0ml to read, 3.0ml of working reagent and 50ul of sample should be used.

Procedure Notes

1. Samples with values above 20 mg/dl should be diluted 1:1 with saline, re-assayed and the result multiplied by two.
2. Lipemic or hemolyzed samples require a serum blank. To prepare a serum blank add 0.02ml (20ul) of sample to 1.0ml D-water. Mix and read against water at 570nm. Subtract the absorbance reading from the test reading and perform calculation.

- Contamination of glassware with calcium will adversely affect test results. Acid-washed glass or plastic test tubes are recommended.

Calibration

Use an aqueous Calcium Standard (10mg/dl) or an appropriate serum calibrator.

Calculation

$\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Concentration of Std.} = \text{Calcium (mg/dl)}$

Example: If the absorbance of unknown = 0.47, absorbance of standard = 0.50, concentration of standard = 10mg/dl, then:

$$\frac{0.47}{0.50} \times 10 = 9.4\text{mg/dl}$$

NOTE: To correct mg/dl to mEq/L, divide mg/dl value by two.

Quality Control

The validity of the reaction should be monitored by use of normal and abnormal control sera with known calcium concentrations.

Expected Values¹⁶

8.5 - 10.5 mg/dl

Children under 12 usually have higher normal values which fall with aging.

It is highly recommended that each laboratory establish its own reference range.

Performance

- Linearity: 20 mg/dL
- Comparison: A study performed with a similar method yielded a correlation coefficient of 0.994 with a linear regression equation of $y=1.06x-0.41$
- Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
9.4	0.14	1.5	9.4	0.13	1.4
12.9	0.13	1.0	12.7	0.27	2.1

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