POINTE SCIENTIFIC, INC.
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Calcium (Dry) Reagent Set

Intended Use
For the quantitative determination of calcium in serum.

Method History
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.[1]

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.[2,3]

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.[4,5,6] o-cresolphthalein complexone procedures have since gained wide acceptance for the determination of calcium.

The present procedure uses o-cresolphthalein complexone and has been modified to provide a sensitive and stable reagent system. Magnesium interference is prevented by the inclusion of 8-hydroxyquinoline sulfonate. The reagent is provided in a convenient dry powder form.

Principle
Alkaline Medium

Calcium + o-Cresolphthalein Complexone ----------➢

Calcium – Cresolphthalein Complexone Complex (purple color)

Calcium reacts with o-cresolphthalein complexone in an alkaline medium to form a purple-colored complex which absorbs at 570 nm. The intensity of the color is proportional to the calcium concentration.

Clinical Significance [7,8]
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.

Reagents
Concentrations refer to reconstituted reagent.

Calcium reagent: o-cresolphthalein complexone 0.1mM, 8-hydroxyquinoline sulfonate 8.98mM, Buffer 100mM, pH10.1±0.1, nonreactive stabilizers and fillers with Sodium Azide 0.01% as preservative.

Reagent Preparation
Reconstitute reagent with the volume of distilled water stated on the vial label, swirl to dissolve.

Reagent Storage
1. Store reagent at 2-8°C.
2. Reconstituted reagent is stable for five days at room temperature and 30 days at 2-8°C.

Reagent Deterioration
Do not use if:
1. Moisture has penetrated the vial and caking has occurred.
2. The reagent has an initial absorbance greater than 0.600 versus a water blank at 570 nm.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagent may be irritating to the skin. Avoid contact. Flush with water if contact occurs.
3. Reagent contains Sodium Azide (0.01%) as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to avoid azide build up.

Specimen Collection and Storage
1. Fresh, unhemolyzed serum is the preferred specimen.
2. Heparinized plasma may also be used.
3. Anticoagulants other than heparin should not be used.
4. Remove serum from clot as soon as possible since red cells can absorb calcium.[10]
5. Older serum specimens containing visible precipitate should not be used.[11,12]
6. Serum calcium is stable for 24 hours at room temperature, one week at 2-8°C, and up to five months frozen and protected from evaporation.[13]

Interferences
1. Substances that contain or complex with calcium cause inaccurate results.
2. Bilirubin up to 20mg/dL does not interfere.
3. Severe hemolysis or marked lipemia may cause elevated results. A serum blank should be run for greatest accuracy. (See Procedure Notes)
4. For a comprehensive list of interferences see Young, et al.[14]

Materials Provided
Calcium Reagent.

Materials Required but not Provided
1. Accurate pipetting devices.
2. Timer.
3. Acid-washed glass or plastic test tubes/rack
4. Spectrophotometer with ability to read at 570 nm. (550-580nm).

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Reconstitute reagent according to instructions.
2. Label test tubes: “Blank”, “Standard”, “Control”, “Sample”, etc.
3. Pipette 1.0 ml of reagent into each tube.
4. Add 0.025 ml (25ul) of sample to respective tubes. Mix and let stand at room temperature for at least one minute.
5. Zero spectrophotometer with blank at 570nm.
6. Read and record absorbances of all test tubes.
7. See “CALCULATIONS” section to determine results.

Procedure Notes
1. Final color is stable for thirty minutes.
2. Samples with calcium above 20 mg/dL should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
3. Lipemic or hemolyzed samples require a serum blank. Add 25ul of sample to 1.0 ml distilled water. Read against water at 570nm and subtract the absorbance reading from the test absorbance.
4. Contamination of glassware with calcium will adversely affect test results. Acid-washed glass or plastic test tubes are recommended.

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Limitations
Samples with calcium values exceeding 20mg/dL should be diluted with an equal volume of distilled water, the assay repeated, and the result multiplied by two.
Severely lipemic or hemolyzed samples should be run with a serum blank for greatest accuracy. See "Procedure Notes".

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

Calculations
$$\frac{\text{Abs. of Sample}}{\text{Abs. of Standard}} \times \text{Concentration of Std.} = \text{Calcium (mg/dL)}$$

Example: If the absorbance of sample = 0.90, absorbance of standard = 0.95, concentration of standard = 10 mg/dL, then:

$$\frac{0.90}{0.95} \times 10 = 9.5 \text{ mg/dL}$$

NOTE: To convert mg/dL to mEq/L, divide mg/dL value by two.

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

Expected Values[15]
8.5 – 10.4 mg/dL
It is strongly recommended that each laboratory establish its own normal range.

Performance
1. Linearity: 20 mg/dL
2. Comparison: A study performed using another o-cresolphthalein procedure yielded a correlation coefficient of 0.996 with a regression equation of y=0.95 x + 0.47.
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
9.1	0.08	0.9	8.9	0.09	1.0
12.0	0.13	1.1	12.1	0.24	2.0

4. Sensitivity: Using the manual procedure described, 1mg/dL calcium will produce an absorbance of approximately 0.090.

References

1. Cali, J.P., et al, N.B.S., Sp. Publication 260:36 (1972).
2. Connerty, H.V. and Biggs, A.R., Clin. Chem. 11:716 (1965).
3. Gindler, E.M., and King, J.D., Am. J. Clin. Path. 58:376 (1972).
4. Connerty, H.V. and Biggs, A.R., Am. J. Clin. Path. 45:290 (1966).
5. Gitelman, H.J., Anal. Biochem. 18:521 (1967).
6. Baginski, E.S., et al, Clin. Chem. Acta 46:49 (1973).
7. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.149 (1984).
8. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods, Philadelphia, W.B. Saunders, p.149 (1984).
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. Tayeau, F., et al, Bull. Soc. Pharm. Bordeaux, 95:206 (1956).
13. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, Hagerstown (MD), Harper & Row, p.669 (1974).
14. Young, D.S. et al, Clin. Chem. 21:1D (1975).
15. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p. 1208 (1984).

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Rev. 5/03 P803-C7508-01