

Calcium (CPC)

1.0 INTENDED USE

This reagent is intended for the quantitative determination of total calcium concentration in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

The source of color reagents presently used for the colorimetric determination of calcium came from the early titrimetric determinations of Pollard (10.1) and Schwarzenbach (10.2). The primary source of interference with calcium color reactions has been magnesium which also reacts to some extent with the same complexones. In order to obviate this problem Connerty and Briggs (10.3) described a procedure in which cresolphthalein complexone (CPC) was used to determine calcium while 8-hydroxy-quinoline was employed to complex with magnesium effectively removing it from the system. Zak et. al.(10.4) have recently reviewed calcium methodologies and have recommended the direct cresolphthalein complexone procedure for serum calcium. This procedure for serum calcium is a modification of the Connerty and Briggs procedure employing CPC as a color developer and 8-hydroxy-quinoline to mask the presence of magnesium.

2.2 TEST PRINCIPLE

Cresolphthalein complexone (CPC) reacts with calcium to form a purple colored complex.

Cresolphthalein Complexone + 2Ca^{++} \rightarrow CPC (Ca^{++})₂

The formation of the purple colored complex causes an increase in absorbance at 570 nm which is directly proportional to the concentration of calcium in the sample.

2.3 CLINICAL SIGNIFICANCE (10.6)

The normal calcium concentration of serum is maintained by hormones in the parathyroid gland. Decreased levels occur in hypoparathyroidism, vitamin D deficiency, rickets, osteomalacia and renal tubular acidosis. Increased levels are found in hyperparathyroidism, vitamin D intoxication, and are associated with neoplasms, especially those of bone.

A significant fraction of serum calcium is bound to protein. Hyperproteinemia is associated with an increased level of serum calcium and hypoproteinemia is associated with a decreased level of serum calcium.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Heparinized plasma may also be used. Plasma prepared using EDTA, oxalate, citrate, which function by removal of calcium, obviously must not be used.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 μl . Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, the serum sample can be stored refrigerated (2-8°C) for up to 7 days.

4.0 MATERIALS (2 X 125 ml) (4 X 125 ml)

Reagents necessary for the determination of calcium are included in the kit.

4.1 REAGENT

- 4.1.1 Calcium Color Reagent contains 0.10 mM cresolphthalein complexone and 17.2 mM 8-hydroxyquinoline.
- 4.1.2 Calcium Color Developer contains 0.6 M AMP buffer and a surfactant.
- 4.1.3 Standard/Control/Calibrator

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

The working reagent is prepared by mixing equal volumes of the color reagent and color developer.

4.4 REAGENT STORAGE AND STABILITY

The reagent is stable at room temperature (18-26°C) until the expiration date on the label. The working reagent is stable for 7 days at room temperature.

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 570 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 570 nm.
- 4.5.3 Test tubes capable of holding 3 ml.
- 4.5.4 Pipettes capable of delivering 2.5 ml and 25 µl.
- 4.5.5 Timer for 5 minute incubation.
- 4.5.6 Distilled or deionized water.
- 4.5.7 Calibrator
- 4.5.8 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	570 nm
Temperature	18-26° C or 37° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	5 minutes
Sample volume	25 µl
Reagent volume	2.5 ml
Total volume	2.525 ml
Sample to reagent ratio	1/100

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 570 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The calcium assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

- 5.4.1 Prepare the required volume of working reagent. (See 4.3 Reagent Preparation section.)
- 5.4.2 Into separate calcium free test tubes pipette 25 µl of distilled water, calibrator, or serum to be assayed.
- 5.4.3 Add 2.5 ml of working reagent and mix.
- 5.4.4 Incubation for 5 minutes at the desired temperature and determine the absorbance of the calibrator (As) and of each serum (A) at 570 nm using the distilled water sample as the reagent blank.

5.5 PROCEDURE NOTE

The color is stable for 1 hour.

A major source of difficulty with the assay of calcium is contaminated glassware employed in the performance of the test. Many detergents and water supplies contain calcium and incompletely rinsed containers used in this test will lead to inaccurate results.

5.6 CALCULATION AND RESULTS

$$\text{Calcium (mg/dl)} = \frac{A}{A_s} \times \text{concentration of calibrator}$$

A = absorbance of sample, A_s = absorbance of calibrator
Example:

$$\text{Calcium concentration} = \frac{.359}{.405} \times 10 \text{ mg/dl} = 8.9 \text{ mg/dl}$$

with A = .359 and A_s = .405, concentration of calibrator = 10 mg/dl

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES

The range of expected values is: 8.7 - 10.7 mg/dl

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.7)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

Any substance which either chelates calcium or contains calcium will interfere with the assay.

Young et al. (10.5) have published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that for serum calcium.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The calcium assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 10 replicates of 2 control sera were run.

Mean (mg/dl)	SD (mg/dl)	CV (%)
9.6	± 0.06	0.6
13.8	± 0.11	0.8

Between-Run

In this study, 10 runs were made on a pooled serum sample.

Mean (mg/dl)	SD (mg/dl)	CV (%)
9.5	± 0.10	1.1

9.2 CORRELATION

A correlation study was done comparing this method (y) with a similar calcium o-cresolphthalein method (x). The study yielded a regression curve of $y = 1.045x - 0.0812$.

9.3 LINEARITY

This procedure is linear through 20 mg/dl beyond which the specimen should be diluted 1 to 1 with 0.9% saline. Reassay the specimen and multiply the results by 2.

10.0 REFERENCES

- 10.1 Pollard, F.H., J.V., Analyst 81, 348-353 (1956).
- 10.2 Schwarzenbach, G., Analyst 80, 713-729 (1955).
- 10.3 Connerty, H.V., Briggs, A.R., Am. J. Clin. Path. 45, 290-296 (1966).

- 10.4 Zak, B., Epstein, E., Babinski, E.S., *Annals of Clinical and Laboratory Science* 5, 195-215 (1975).
- 10.5 Young, D.S., *Effects of Drugs on Clinical Laboratory Tests*, 3rd ed., Washington DC, AACC Press (1990).
- 10.6 Todd, Sanford and Davidsohn, *Clinical Diagnosis and Management by Laboratory Methods*, Edited by Henry, J.B., W.B. Saunders Company, Philadelphia, 1969, p.575.
- 10.7 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; *JAMA*, Feb. 2, 1990; Vol 263, No.5, p.704

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