TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

CALCIUM
COLORIMETRIC METHOD
TC MATRIX

INTENDED USE
For the direct, colorimetric determination of calcium in human serum or urine on TC Matrix analyzers.

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 CONTENTS:
Each kit contains: Three Calcium Reagent 1(3×40 ml)
Three Calcium Reagent 2 (3×40 ml)
Instruction Insert.

REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
When reconstituted as directed, the reagent for calcium contains the following:
O-Cresolphthalein Complexone: 0.14 mM
8-Hydroxyquinoline: 13 mM.
Diethylamide 363 mM:
Potassium Cyanide: 2 mM
Non-reactive ingredients, and stabilizers in both reagents 1 and 2.

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 (1) and (2) may be irritating to skin. Avoid contact.
3. Reagent (2) contains cyanide and should **NOT BE PIPETTED BY MOUTH.**

REAGENT STORAGE AND STABILITY
1. All reagents should be stored at room temperature (15 - 30°C).
2. Combined reagent (1 and 2) 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.

CALIBRATION
1. Calibrator required: TECO MULTI Calibrator.
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

Note: Refer to the TC Matrix manual for further instructions on calibrating the instrument

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

TECO MULTI Calibrator
At least two levels of control material.

PRECAUTIONS:

1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety in Microbiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.
4. **Recommended to test alone.**

PROCEDURES

TEST NAME:	CA	R1:	180
TEST NO.		R2:	180
FULL NAME:	Calcium	SAMPLE VOLUME:	7
REFERENCE NO.:		R1 BLANK:	/
ANALY.TYPE:	Endpoint	MIX REAG. BLANK:	0.1 - 1.5
PRI. WAVE :	578 nm	CONCENTRATION:	/
SECON. WAVE:	/	LINEARITY LIMIT:	0 - 20
TREND:	Ascending	SUBSTRATE LIMIT:	/
REACT. TIME:	0 - 4	FACTOR:	/
INCUBATE TIME:	3	PROZONE CHECK:	/
UNIT:	mg/dl	Q1: / Q2: /	Q3: / Q4: /
PRECISION:	0.1	PC: /	ABS.: /
Calibration Type: Calibrate+Reag.Blank		Calibration Rule: One-point linear	

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.

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 = 0.97 x - 0.48.
3. *Precision:*

	Within Run	
Mean (mg./dl)	S.D.	C.V. (%)
8.8	0.37	3.1%
12.3	0.23	1.4%

	Run to Run	
Mean (mg./dl)	S.D.	C.V. (%)
8.8	0.24	2.4%
12.3	0.22	1.7%

REFERENCES

1. Faulker, W.R. and Meites,S.: *Selected Methods for the Small Clinical Chemistry Laboratory,* Washington, D.C. p. 125 (1982).
2. Cali, J.P., et al.: *A Reference Method for the Determination of Calcium in Serum* N.B.S., sp. publication, 260:32 (1972).
3. Andregg, G., et al.: *HeIv. Chim Acts.* 37: (1954).
4. Schwarzenbach, G.: *Analys.* 80:713 (1955).
5. Gitelman, H.: *Anal. Biochem.* 20:5521 (1967).
6. Richterich, R.: *Clinical Chemistry:* Theory and Practice. Academic Press, New York, p. 304 (1969).
7. Peters, J.P., Van Slyke, D.D.: *Quantitative Clinical Chemistry.* vol. 2. Baltimore, Williams and Wilkins, (1932).
8. Chen, P.S., et al.: *Anal. chem.* 26:1967 (1954).
9. Tayeau, F., et al.: *Bull Sec. Pharm. Bordeaux* 95:206 (1956).
10. Kopito, *L., et al.: N. EngI. J. Med.* 2730:113 (1965).
11. Henry, R.J., et al.: *Clinical Chemistry: Principles and Technics.* Harper and Row, Hagerstown, M.D.p. 669 (1974).

C503-580TM: 02/08

Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.

Authorized Representative:
Emergo Europe
P.O. Box 149
4300 AC Zierikzee
The Netherlands