CALCIUM COMPLEXONE

Colorimetric determination of calcium in biological liquids

TEST SUMMARY

o-cresolphtalein complexone combines with calcium at alkaline pH to form a red-violet complex, which absorbance is measured at 575 nm. The reaction has high specificity for calcium and interference from magnesium is avoided due to selective complexing agent.

SAMPLES

Serum (preferred), plasma heparinate. Do not use citrate, oxalate or EDTA as anticoagulant.
Total calcium is stable 7 days at 2-8°C and for several months at –20°C.
Urine samples should be collected in 20-30 ml di HCl 6M per 24/h specimen (1 – 2 ml for spontaneous urine) in order to prevent calcium salt precipitation.
Dilute urine 1:2 with distilled water and mutiply by 2 the obtained results.

REAGENTS

Reagent A:	o-cresolphtalein complexone 0.14 mM, 8-quinolinol 26 mM, chloridical acid pH 1.20.
Reagent B:	AMP buffer M pH 11.00, surfactants.
Standard:	calcium solution 10 mg/dl.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general “Good Laboratory Practice” (GLP) guidelines.

REAGENTS PREPARATION

Mix some Reagent A with some Reagent B.
Reagents are stored at 15-25°C until expirtion date away from light source or 60 days after first opening.
Stability of working reagent: 14 days at 2-8°C, 7 days at room temperature, well closed.

PROCEDURE

Kind of analysis:	End point
Reading time:	2 minutes
Wavelength:	575 nm (570-580)
Temperature:	25, 30 or 37°C
Lightpath	1 cm
Zero:	Reagent blank

Reagents	Blank	Standard	Sample
Distilled Water	20 µl	--	--
Standard	--	20 µl	--
Sample	--	--	20 µl
Reagent	1 ml	1 ml	1 ml

CALCULATION

Serum/Plasma Calcium (mg/dl)

(A sample/A standard) x 10

Spontaneous urine Calcio (mg/dl)

(A sample/A standard) x 10 x 2

24h urine Calcium (mg/24h)

(A sample/A standard) x 10 x 2 x diuresis (dl)

EXPECTED VALUES

Serum/plasma

	8.6 – 10.3 mg/dl	(2.15 – 2.57 mmol)

Urine 24h

Men:	≤ 300 mg/24h	(7.49 mmol/24h)
Women:	≤ 250 mg/24h	(6.24 mmol/24h)

Every laboratory should establish own reference intervals in accordance with own population.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/ QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

QN 0050 CH **10 x 5 ml**
Control Sera normal values

QP 0050 CH **10 x 5 ml**
Control Sera pathological values

TEST PERFORMANCE

Precision

Intra-assay (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	9.770	0.051	0.520
Sample 2	13.746	0.062	0.450

Inter-assay (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	9.730	0.086	0.890
Sample 2	13.760	0.120	0.850

Sensibility/limit of detection
The method is able to discriminate up to 0.1 mg/dl.

Linearity
The method is linear up to 20 mg/dl.
If the value is exceeded, is suggested to dilute the sample 1+9 with physiological solution and perform again the test, multiplying the results by 10.

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 50 samples of serum:

Calcium LTA = x
Calcium competitor = y
n = 50

y = 1,0009 - 0,01277x r = 0,99534

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 20 samples of urine:

Calcium LTA = x
Calcium competitor = y
n = 20

y = 1,01488x - 0,17995 r = 0,9953

Interferences
No interference was observed in presence of:

hemoglobin	≤ 350 mg/dl
bilirubin	≤ 40 mg/dl
lipids	≤ 500 mg/dl

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC00800	(600 TESTS)	
Reagent A	3 x 100 ml	(liquid)
Reagent B	3 x 100 ml	(liquid)
Standard	1 x 5 ml	(liquid)

REFERENCES

Zak B., Epstein E., Babinski E.S., Review of Calcium Methodologies, Annals of Clinical ad Laboratory Science 5, 195-212 (1975).
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

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SYMBOLS

IVD Only for IVD use
LOT Lot of manufacturing
REF Code number
Storage temperature interval
Expiration date (year, month)
Warning, read enclosed documents
Read the directions
Biological risk

Mod. 01.06 (ver. 4.3 - 04/03/2006)

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