

# CALCIUM

CA 0505 CH

4 x 125 ml

## SUMMARY OF TEST

In human body, circulating calcium is used for several functions, in skeletal metabolism as well as in neuromuscular function and in hemostasis.

Serum calcium is affected by malabsorption, parathyroidal problems, bones disease, circulating albumin diseases.

Typical variations:

greater than normal levels in hyperparathyroidism, metastatic bone tumor, milk - alkali syndrome, multiple myeloma, Paget's disease, sarcoidosis, vitD intoxication; lower than normal levels in hypoparathyroidism, malabsorption, osteomalacia, pancreatitis, renal failure, vitD deficiency.

The amount of calcium excreted into the urine reflects intestinal absorption, skeletal resorption, and renal tubula filtration and reabsorption. Under fasting conditions, the intestinal and renal components are relatively fixed and calcium excretion (mg/dl of glomerular filtrate) in the fasting state is used to assess the skeletal components.

In the present method, o-cresolphthalein complexone as a calcium binding reagent shows high specificity and sensitivity in formulation conditions and therefore a reliable, simple and fast methodology has been developed.

## PRINCIPLE OF THE METHOD

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

## KIT COMPONENTS

### For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label at 15-25°C.

Keep away from direct light sources.

### Reagent A: 2 x 125 ml (liquid) blue cap

Composition: CPC 0.14 mM, 8-quinolinol 26 mM, HCl pH 1.20

### Reagent B: 2 x 125 ml (liquid) red cap

Composition: AMP buffer 1 M pH 11.00, surfactant.

### Standard: calcium solution 10 mg/dl - 5 ml

Store all components at 15-25°C.

## MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micro-pipettes. Glass or high quality polystyrene cuvettes. Saline solution.

## REAGENT PREPARATION

Mix equal quantities of both reagents A and B.

Stability of working reagent: 14 days at 2-8°C and 7 days at room temperature, well closed.

Stability of unopened vials: up to expiration date on labels at 15-25°C.

Stability since first opening of vials: ≥ 60 days at 15-25°C.

## PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

## SPECIMEN

Serum (preferred), plasma heparinate. Do not use citrate, oxalate and EDTA as anticoagulant.

Total calcium is stable 7 days at 2-8°C and for several months when frozen at -20°C.

Urine specimens should be collected in 20 to 30 ml of HCl 6M per 24/h specimen (1-2 ml for random urine) in order to prevent calcium salt precipitation.

Dilute sample urine 1:2 with redistilled water and multiply results by two.

## TEST PROCEDURE

Wavelength: 575 nm (allowed 570 ÷ 580 nm)  
Lightpath: 1 cm  
Temperature: 25, 30 or 37°C

dispense:	blank	standard	sample
reagent	3 ml	3 ml	3 ml
water	50 µl	-	-
standard	-	50 µl	-
sample	-	-	50 µl

Mix, incubate at 25, 30 or 37°C for 2 minutes.  
Read absorbances of standard (As) and samples (Ax) against reagent blank.

## RESULTS CALCULATION

serum/plasma sample:

calcium mg/dl = Ax/As x 10 (standard value)

urine sample:

calcium mg/dl = Ax/As x 10 x 2 (standard value and dilution factor)

24 hours urine sample:

calcium mg/24h = Ax/As x 10 x 2 x urine volume (standard value, dilution factor and diuresis in decilitres)

## EXPECTED VALUES

serum/plasma: 8.6 - 10.3 mg/dl (2.15 - 2.57 mmol/l)  
urine (men): up to 300 mg/24h (7.49 mmol/24h)  
urine (women): up to 250 mg/24h (6.24 mmol/24h)

Each laboratory should establish appropriate reference intervals related to its population.

## QUALITY CONTROL AND CALIBRATION

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

**QN 0050 CH QUANTINORM CHEMA 10 x 5 ml**  
with normal or close to normal control values

**QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml**  
with pathological control values.

If required, a multiparametric, human based calibrator is available:

**AT 0030 CH AUTOCAL H 10 x 3 ml**

Please contact Customer Care for further information.

## TEST PERFORMANCE

### Linearity

the method is linear up to 20 mg/dl.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

### Sensitivity/limit of detection (LOD)

the limit of detection is 0.1 mg/dl.

### Interferences

no interference was observed by the presence of:

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

### Precision

intra-assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	8.99	0.08	0.90
sample 2	14.50	0.18	1.20

inter-assay (n=20)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	8.96	0.21	2.40
sample 2	14.72	0.27	1.80

### Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Calcium Chema = x  
Calcium competitor = y  
n = 96

y = 0.95x + 0.158 mg/dl r = 0.957

## WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

## REFERENCES








Zak B., Epstein E., Babinski E.S., Review of Calcium Methodologies, Annals of Clinical and Laboratory Science 5, 195-212 (1975).

Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

## MANUFACTURER

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## SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions