BIOLABO
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MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

CALCIUM CPC method

Reagent for quantitative determination of calcium in human serum and plasma or urines.

REF 80004 **R1** 1 x 200 mL **R2** 1 x 200 mL **R3** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
Fax: (33) 03 23 256 256
support@biolabo.fr

CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

Total Calcium exists in 3 physiochemical states in plasma, of which approximately 50 % is free or ionised calcium, 40 % is bound to plasma proteins, and 10 % are bound with small anions.
The level of serum calcium may be affected by intestinal malabsorption, by alterations in plasma proteins level, especially albumin, which should be measured concurrently with calcium.
Hypercalcemia is found in hyperparathyroïdism, multiple myeloma, bone and parathyroïdal neoplasms and in states with bones demineralisation.
Hypocalcemia is encountered in hypoparathyroïdism and in several cases of necrosis and acute pancreatitis.

PRINCIPLE (4)

Moorehead and Briggs derived CPC (O-Cresol Phtalein Complexone) method allows to determinate total Calcium concentration in serum, plasma or urines.
In alkaline solution CPC reacts with calcium to form a dark-red coloured complex which absorbance measured at 570 nm is proportional to the amount of calcium in the specimen.

REAGENTS

R1 CALCIUM
Buffer

Amino-2-methyl-2-propanol-1 pH 11.0 at 20°C	1.70	mol/L
Hydrochloric acid	210	mmol/L

EUH210: Material safety data sheet available on request

P302+352: IF ON SKIN: Wash with soap and water

P305+351+338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing

R2 CALCIUM
Dye

0-cresolphtalein complexone (CPC)	78	µmol/L
Hydroxy-8-Quinoline	3.36	mmol/L
Hydrochloric acid	25	mmol/L

R3 CALCIUM
Standard

Calcium	10	mg/dL (2.5 mmol/L)

According to 1272/2008 regulation, these reagents are not classified as dangerous

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).
- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.

All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

REAGENTS PREPARATION

Working reagent (WR):
In a carefully cleaned container with HCl 0.1 N and well rinsed with demineralised water, mix 1 volume of R1 and 1 volume of R2.
Reagents may also be added separately.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 18-25°C, reagents are stable when stored and used as described in the insert:

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Transfer requested quantity, well recap vials and store at 18-25°C,
- Separate reagents (R1 and R2) are stable at least 3 months
- Working reagent (R1+R2) is stable for 1 day when free from contamination.
- Discard reagent if cloudy or if reagent blank at 570 nm is > 0.400.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Serum or heparinised plasma:
Do not use citrate, oxalate or EDTA. Blood obtained on fasting patient with minimal venous occlusion and without exercise or after restoring circulation at least for 1 minute.

24 h Urines:
Acidify after collection with 20 to 30 mL HCl 6 N to dissolve calcium salts.
Dilute (1 + 2) with distilled water before performing the test.

Total calcium is stable in serum for:
- at least 7 days at 2-8°C.
- 6 months at -20°C.

Long-term freezing may lead to associated evaporation, lyophilisation or co precipitation with fibrin (i.e. heparinised plasma) or lipids.

LIMITS (3)

Handle with care specimens, calibrators and controls to avoid contamination by environmental calcium. Use disposable tubes and cuvettes and glassware cleaned with HCl 0.1 N, well rinsed with demineralised water.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. EDTA Solution 10 mM for serum blank
3. Spectrophotometer or Biochemistry Clinical Analyzer

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- REF 95012 Urinary controls
- External quality control program

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

CALCIUM, total in serum:

Population	mg/dL	mmol/L
Premature	6.2-11.0	[1.55-2.75]
0-10 days	7.6-10.4	[1.90-2.60]
10 days –24 months	9.0-11.0	[2.25-2.75]
24 months –12 years	8.8-10.8	[2.20-2.70]
12 years -18 years	8.4-10.2	[2.10-2.55]
18-60 years	8.6-10.0	[2.15-2.50]
60-90 years	8.8-10.2	[2.20-2.55]
> 90 years	8.2-9.6	[2.05-2.40]

TOTAL CALCIUM in 24 h Urines: < 300 mg/24 h (< 7.5 mmol/24 h)

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES at 37°C on KENZA 240TX

Linearity Range: between 5.2 and 20 mg/dL

Detection limit: approx. 0.04 mg/dL

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	4.71	10.61	13.42
S.D. mg/dL	0.07	0.17	0.17
C.V. %	1.6	1.6	1.3

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	4.80	10.39	13.27
S.D. mg/dL	0.09	0.15	0.21
C.V. %	1.9	1.4	1.6

Comparison studies with commercially available reagent:

Realised on Automated analyzer with serum specimens between 5.19 and 16.42 mg/dL (n=93)

y = 1.0249x - 0.23654 R= 0.9985

Analytical Sensitivity: approx. 0.093 abs for 1 mg/dL

Interferences:

Turbidity	Positive interference from 0.093 OD
Total bilirubin	No interference up to 534 µmol/L
Direct bilirubin	No interference up to 406 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1089 mg/dL
Haemoglobin	Positive interference from 109 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 8 days

Calibration Stability: 8 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

CALIBRATION (6)

- REF 95015 BIOLABO Multicalibrator traceable *to SRM 909c*
- Standard (R3)

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 570 nm

Temperature: 37°C

Temperature should be held constant as the absorbance of the dye is temperature sensitive.

	Automated analyzer	Manual procedure
Reagents	120 µL R1 120 µL R2	WR :1000 µL
Standard, Controls, Specimen	6 µL	25 µL
Mix well. Incubate for 5 minutes at room temperature. Read absorbance at 570 nm (550-590) against reagent blank. The coloration is stable for 1 hour away from light		

Notes:

1- For urines, use standard of the kit to calibrate (do not dilute) and control with REF 95012 (to be treated as patient's urines)

2- Performances and stability data's have been validated with serum on KENZA 240TX and KENZA 450TX

3-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.

4- Applications proposal are available on request

5- Haemolysis, cloudy or icteric sera:

- Manual Procedure: perform a specimen blank by adding a drop (25 µL) of EDTA solution 10 mM in assay and blank reagent tubes, mix and re-read. Deduct this value from the previously reading obtained for the specimen.
- Automated procedure: Bichromatic analysis: the 2nd wavelength may be 500, 650 or 700 nm.

CALCULATION

Calculate the result as follows:

Serum or plasma:

$$\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \times \text{Standard concentration}$$

Urines:

Multiply the above result by dilution factor 3

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1395-1406, p.1435-1439.*

(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 202-207*

(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-115 to 3-124*

(4) *MOOREHEAD W.R., BRIGGS H.G., Clin. Chem., (1974), 20, p.1458-1460*

(5) *W. L. CLARK, E L BAGINSKI, S S MARIE, et B. ZAK, Spectrometric Study of a direct determination of Serum Calcium, Microchem. J.,20, (1975), p.22-32.*

(6) *SRM:Standard Reference Material ®*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with | H2O Demineralized water | Biological hazard

Made in France

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