DIRECT FREE CALCIUM - CPC LR liquid reagent

Colorimetric Method F. Amato

REF C1810130A 1x30 ml

CE IVD For in vitro medical device

Use

Free Calcium Test is intended for the quantitative in vitro diagnostic determination of free calcium (ionized calcium) in human serum or plasma. Colorimetric CPC o-cresolphtalein complexone method.

Summary

Free Calcium measurements are used in the diagnosis and monitoring in the laboratory for the treatment of disorders of calcium metabolism.

Principle

End point analysis. Under alcaline condictions O-cresolphtalein complexone (CPC) reacts with calcium ions and magnesium to form a purple complex :the magnesium interference is inhibited by 8-hydroxyquinoline presence. Absorbance measurements are taken at 570 nm. The increase in absorbance due to purple complex is directly proportional to the calcium concentration in the tested sample, not linked to the proteins.

Reagents

R1	Alcaline Buffer	n.d.
R2	O-cresolphtaleina complexone	0.16 mmol/l
	8-hydroxyquinoline	9.0 mmol/l
R3 (C)	Na_2EDTA	40.0mmol/l

Reagents preparation

Reagents are liquid and ready to use. About using as monoreagent ("sample-starter" procedure) pour the reagent **R2** into **R1** vial . For a lower quantity add to every 2 ml of **R1** reagent, 1 ml of **R2** reagent.

Storage and Stability

- Store the kit at 15-25°C.
- After opening, the vials **R1**, **R2** are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (**R1**+ **R2**): 1 day at 15-25°C.

Precaution in use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with caution, according to good laboratory practice.

Waste Management

Please refer to the local legal requirements.

Specimen collection and Preparation

- Serum or plasma. (Monoiodioacetate F- Li eparina
- Do not use samples with haemolysis.
- Do not use anticoagulants as EDTA, oxalate, citrate or fluoride.
- Avoid venous standstill. The use of tourniquet can rise the calcium level in the withdrawal in (0.6 mmol/l) as well.
- After picked up,(anaerobial way) serum and plasma must be separated, as soon as possible, from red cells to avoid calcium absorption by red cells.
- The Free calcium must be tested as soon as possible. The Free calcium is stable in the serum or plasma 1 day at 2-8°C or 6 months at -20°C.

Note

- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedures

Wavelength	λ: 578 (570-600) nm
Working Temperature	37°C°
Optical path	1 cm
Reaction	"end point" (increasing)

- Monoreagent procedure "sample starter"

	BLANK	STD	SAMPLE
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	100 µl	--	--
Sample	--	--	100 µl
Standard	--	100 µl	--

Mix, then incubate for 10' at 37°C. Measure the absorbance of the sample (EC) and standard (ESTD) against the reagent blank.

- Bireagent procedure "cromogeno starter"

	BLANK	STD	SAMPLE
Reagent R1	700 µl	700 µl	700 µl
Distilled Water	100 µl	--	--
Sample	--	--	100 µl
Standard	--	100 µl	--
Mix then incubate at 15-25°C for about 1', then add :			
Reagent R2	350 µl	350 µl	350 µl

Mix then incubate at 10' a 37°C. Measure the absorbance of the sample (EC) and standard (ESTD) against the reagent blank.

Calculation

Calcium [mg/dl] o [mmol/l] = EC/ESTD x Conc. STD

The reagent performances are related to 37°C, and 578 nm.

Conversion Factor

Calcium [mg/dl] x 0.2495 = Calcium [mmol/l]
Calcium [mg/dl] x 0.4990 = Calcium [mEq/l]

Reference Values

Serum - plasma
Adult 1,00 – 1,30 mmol/L
Children 1,00 – 1,30 mmol/L

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Linearity

Reaction is linear up to a concentration di 3.0 mmol/L con a range of 0.01-4,50 mmol/L .

"Intra-Assay" precision (within -Run)

Determined on 20 samples for each control(N-H) (Normal- High). Results:

MEDIA (mg/dl)	N = 1.08	P = 1.75
D.S.	N = 0.011	P = 0.021
C.V.%	N = 1.02	P = 1.22

"Inter-Assay"precision (between-Run)

Determined on 20 samples for each control(N-H) (Normal- High). Results:

MEDIA (mg/dl)	N = 1.08	P = 1.75
D.S.	N = 0.011	P = 0.021
C.V.%	N = 1.02	P = 1.22

Analytical Sensibility

The test sensitivity in terms of detection limit is: 0.05 mmol/l

Correlation

An I.S.E. method taken as reference, correlated on 20 samples with the tested reagent, has given a correlating factor : **r = 0.94**

y = 0.933x + 0.8075

Interferences

No interference was observed by the presence of:

Bilirubin	≤	5 mg/dl
Triglycerides	≤	500 mg/dl
Hemoglobin	≤	200 mg/dl
Magnesium	≤	5 mg/dl
Ascorbic Acid	≤	25 mg/dl

Quality controls

It's necessary, each time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

1) Manzo F., Amato F. : 40° NATIONAL CONGRESS A.I.Pa.C. Bolzano,29/5-1/6 1990, Free Calcium colorimetric, communication.

2) F.Amato: "Free Calcium colorimetric (cCa2+): principle and application" Acts of Congress FREE CALCIUM ON LABORATORY DIAGNOSTIC : A NEW METHOD PURPUSAL, Palermo 9 giugno 1990;

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	In vitro medical devices
LOT	Batch code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan Production srl

Gesan Production s.r.l.
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