BECKMAN COULTER CE

AU

Instructions For Use

CA
Calcium oCPC

REF OSR6113 4 x 27 mL R1, 4 x 27 mL R2
OSR6213 4 x 50 mL R1, 4 x 50 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Photometric colour test for the quantitative determination of total calcium in human serum, plasma and urine on Beckman Coulter AU analysers.

SUMMARY AND EXPLANATION

Reference[1]

Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease, urolithiasis and tetany (intermittent muscular contractions or spasms).

Total serum calcium is composed of three fractions: free or ionised calcium, 50%; protein bound calcium most of which is bound to albumin with only a small portion bound to globulins, 45%; and complex-bound calcium, mainly to phosphate, citrate, and bicarbonate, 5%. The ionised calcium is physiologically most significant, but has proven difficult to assay directly. It may be estimated from total calcium given knowledge of the protein content and pH of the blood, which strongly affect the level of ionised calcium.

Calcium ions are important in the transmission of nerve impulses, as a cofactor in several enzyme reactions, in the maintenance of normal muscle contractility, and in the process of coagulation. A significant reduction in calcium ion concentration results in muscle tetany. A higher than normal concentration of calcium ions produces lowered neuromuscular excitability and muscle weakness along with other more complex symptoms.

METHODOLOGY

Reference[2,3]

Calcium ions react with o-Cresolphthalein-complexone in an alkaline medium to form a purple coloured complex. In this method the absorbance of the Ca-oCPC complex is measured bichromatically at 570/660 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

CHEMICAL REACTION SCHEME

$$Ca^{2+} + \text{oCPC} \xrightarrow[\text{pH 10.6}]{\text{8-Hydroxyquinoline}} \text{Ca-oCPC Complex}^{2+} \text{ (purple)}$$

SPECIMEN

TYPE OF SPECIMEN

Serum or heparinised plasma should be promptly separated to avoid the uptake of calcium by erythrocytes. Do not use the following anticoagulants in collecting blood for use in this test: EDTA, Sodium Citrate, Sodium Fluoride or Oxalate.[4]

Stable in serum when stored at 2…8°C for up to 1 week.[5]

Urine[6]: Acidified with 6M HCl. Collect timed 24 hour specimen using standard laboratory procedures.

Store at 2…8°C.

REAGENTS

WARNING AND PRECAUTIONS

Dispose of all waste material in accordance with local guidelines.

Exercise the normal precautions required for handling all laboratory reagents.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Ethanolamine (pH 10.6)	0.375 mol/L
8-Hydroxyquinoline	7.16 mmol/L
o-Cresolphthalein complexone	82.0 µmol/L

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

GHS HAZARD CLASSIFICATION

Calcium R1 — DANGER

H314	Causes severe skin burns and eye damage.
H335	May cause respiratory irritation.
P261	Avoid breathing vapours.
P280	Wear protective gloves, protective clothing and eye/face protection.
P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.

	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Ethanolamine 5 - 10%
Calcium R2	DANGER	
		
		
		
	H314	Causes severe skin burns and eye damage.
	H317	May cause an allergic skin reaction.
	H360	May damage fertility or the unborn child.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
		Hydrochloric Acid < 0.2%
		8-Hydroxyquinoline < 0.5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…25°C. Once open, reagents stored on board the instrument are stable for 30 days.

AU5800: Once open, reagents stored on board the instrument are stable for 21 days.

CALIBRATION

CALIBRATOR REQUIRED

Use System Calibrator Cat. No. 66300 for serum and plasma application and Urine Calibrator Cat. No. B64606 for urine application.

The calcium value of System Calibrator Cat. No. 66300 for serum/plasma application is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 909b Level 1. The calcium value of the urine calibrator B64606 is traceable to NIST SRM 915b.

Recalibrate the assay every day or, when the following occur:

Change in reagent bottle or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

AU5800:

Recalibrate the assay when there is a significant shift in control values or when the following occur:

Change in reagent bottle.

Major preventative maintenance was performed on the analyser or a critical part was replaced.

Absorption of atmospheric CO_2 by the reagent on board the analyzer can impair calibration stability. This effect will vary depending upon the rate of use. Consequently each laboratory should set a calibration frequency in the instrument parameters appropriate to their usage pattern.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used for the serum/plasma application.

Biorad Liquichek Urine Chemistry Controls Cat. No. 397 and 398 or other control materials with values determined by this Beckman Coulter system may be used for the urine application.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analysers automatically compute the calcium concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Serum, plasma[1] — Adults	2.20 – 2.65 mmol/L or 8.8 – 10.6 mg/dL
Serum, Children 0 – 10 day[7]	1.90 – 2.60 mmol/L or 7.6 – 10.4 mg/dL
Serum, Children 2 – 12 year	2.20 – 2.70 mmol/L or 8.8 – 10.8 mg/dL
Urine[1]:	
24h urine	2h urine
Female < 6.2 mmol (250 mg)	Male and Female ≤ 0.57 mmol/mmol (0.2g/g) of creatinine
Male < 7.5 mmol (300mg)	
Male and Female ≤ 0.1 mmol (4 mg)/kg of body weight	
Small Children: ≤ 0.8 g/g creatinine	

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

LIMITATIONS

Care should be taken when interpreting calcium results from patients who have received gadolinium containing contrast medium within the previous 24 hours, especially if the patient has impaired renal function.[8,9,10] Such samples should be assayed using non-colorimetric techniques e.g. ion selective electrodes or emission spectroscopy. If non-colorimetric assays are unavailable, samples should be drawn prior to administration of such contrast media.

INTERFERENCES

Results of serum studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 3% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 3% up to 5.0 g/L haemoglobin
Lipemia:	Interference less than 10% up to 1,000 mg/dL Intralipid

Results of urine studies conducted to evaluate the susceptibility of the method to interference were as follows:

Ascorbate :	Interference less than 3% or 0.7 mmol/L up to 50 mg/dL ascorbate
Magnesium:	Interference less than 3% or 0.7 mmol/L up to 40 mg/dL magnesium

Refer to Young[11] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0 – 4.5 mmol/L (0 – 18 mg/dL) for serum and plasma. The test is linear within a concentration range of 0 – 10 mmol/L (0 – 40 mg/dL) for urine.

SENSITIVITY

The lowest detectable level in serum on an AU640 analyser was estimated at 0.03 mmol/L.

The lowest detectable level in urine on the AU2700 was estimated at 0.10 mmol/L.

The lowest detectable level represents the lowest measurable level of calcium that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this Calcium oCPC OSR6113 assay on the AU640 against a flame photometry method. Results of linear regression analysis were as follows:

y = 0.986x + .002	r = 0.985	n = 106	Sample range = 1.37 – 3.57mmol/L

Patient urine samples were used to compare this Calcium oCPC OSR6113 assay on the AU2700 against another commercially available calcium assay. Results of linear regression analysis were as follows:

y = 0.971x + .004	r = 0.997	n = 125	Sample range = 0.07 – 7.73mmol/L

PRECISION

The following data was obtained on an AU640 using 3 serum control materials analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
2.27	0.01	0.60	0.04	1.72
2.62	0.02	0.63	0.03	1.14
3.49	0.02	0.51	0.05	1.40

The following data was obtained on an AU640 using 3 urine pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
1.13	0.02	2.02	0.04	3.93
4.42	0.03	0.75	0.06	1.33
9.65	0.05	0.53	0.11	1.17

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
CAO1N	Calcium oCPC (Serum)
CAO1N, CAO1NP	Calcium oCPC (Urine)
CAO1NP	Calcium oCPC (Serum Paediatric)

Setting Sheet Footnotes
User defined
† System Calibrator Cat. No.: 66300

† Urine Calibrator Cat. No: B64606. Ensure relevant value sheet is used.

* Values set for working in SI units (mmol/L). To work in mg/dL multiply by 4.

‡ Depends on usage pattern in the laboratory.

** CAO1N to link with Serum Application, CAO1NP to link with Paediatric Serum Application

** Test Name ‘CA’ to link with Paediatric Serum Application ‘CAP’

REVISION HISTORY

Revised Interferences section.

Preceding version revision history

Added new languages

REFERENCES

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