AU

Instructions For Use

CA
CALCIUM ARSENAZO

REF OSR60117 4 x 15 mL R1
OSR61117 4 x 29 mL R1
OSR66117 4 x 173 mL R1

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 AU Beckman Coulter analysers.

OSR66117 for use on the AU5800, AU2700 and AU5400 systems only.

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]

This Calcium procedure is based on calcium ions (Ca^{2+}) reacting with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenearsonic acid) to form an intense purple coloured complex. In this method the absorbance of the Ca-Arsenazo III complex is measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

Magnesium does not significantly interfere in calcium determination using Arsenazo III.

CHEMICAL REACTION SCHEME

Ca^{2+} + Arsenazo III $\xrightarrow[\text{pH 6.9}]{}$ Ca-Arsenazo III complex (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 and plasma for 3 weeks when stored at 2…8°C and 7 days when stored at 15…25°C.[5]

Urine:[6] Acidified with 6M HCl. Collect timed 24 hr specimen using standard laboratory procedures. Samples with urine pH below 1.5 may result in a negative bias.

Store at 2…8°C.

Stable in urine for 4 days when stored at 2…8°C and 2 days when stored at 15…25°C.[5]

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Imidazole (pH 6.9)	
Arsenazo III	0.02%
Triton X-100	
Preservative	

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.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Calcium Arsenazo	DANGER	
		
		
	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	H360	May damage fertility or the unborn child.
	EUH208	May produce an allergic reaction.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
		Imidazole 1 - 2%
		2-Chloroacetamide < 0.1%

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

REAGENT PREPARATION

The reagent is ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagent is stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagent stored on board the instrument is stable for 90 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.

Change in reagent lot number or significant shift in control values,

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

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 (8.8 – 10.6 mg/dL)
Serum, Children 0 – 10 day[7]	1.90 – 2.60 mmol/L (7.6 – 10.4 mg/dL)
Serum, Children 10 day – 24 months[7]	2.25 – 2.75 mmol/L (9.0 – 11.0 mg/dL)
Serum, Children 2 – 12 year[7]	2.20 – 2.70 mmol/L (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 (0.2 g/g) of creatinine
Male < 7.5 mmol (300 mg)	
Male and Female ≤ 0.1 mmol (4 mg)/kg of body weight	
Small Children: 2.28 mmol/mmol (≤ 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.

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

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-colourimetric techniques e.g. ion selective electrodes or emission spectroscopy. If non-colourimetric 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
Magnesium:	Interference less than 10% up to 4 mmol/L magnesium

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

Ascorbate :	Interference less than 3% up to 50 mg/dL ascorbate
Magnesium:	Interference less than 3% up to 4 mmol/L magnesium

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

PERFORMANCE CHARACTERISTICS

LINEARITY

The test is linear within a concentration range of 1 – 5 mmol/L (4 – 20 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 using serum settings on an AU640 analyser was estimated as 0.01 mmol/L.

The lowest detectable level using urine settings on the AU2700 was estimated as 0.03 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 Arsenazo III reagent OSR61117 on the AU640 against Calcium Arsenazo III OSR6176. Results of linear regression analysis were as follows:

y = 1.003x + 0.015	r = 0.999	n = 105	Sample range = 1.03 – 3.83 mmol/L

Patient urine samples were used to compare this Calcium Arsenazo III reagent OSR61117 on the AU640 against Calcium Arsenazo III OSR6176. Results of linear regression analysis were as follows:

y = 1.000x + -0.038	r = 0.999	n = 94	Sample range = 0.50 – 9.62 mmol/L

PRECISION

The following data was obtained on an AU2700 using 3 serum pools analysed over 20 days.

n = 80	Within-run		Total	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
1.61	0.01	0.57	0.02	0.95
2.52	0.02	0.65	0.03	0.96
4.19	0.02	0.48	0.04	0.94

The following data was obtained on an AU2700 using 3 urine pools analysed over 20 days.

n = 80	Within-run		Total	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
0.11	0.00	2.05	0.00	2.60
5.52	0.06	1.00	0.07	1.21
9.33	0.10	1.09	0.13	1.40

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
CAZ1N	Calcium Arsenazo (Serum)
CAZ1N, CAZ1NP	Calcium Arsenazo (Urine)
CAZ1NP	Calcium Arsenazo (Serum Paediatric)
CAZ1NP	Calcium Arsenazo (Urine 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.

** CAZ1N to link with Serum Application, CAZ1NP to link with Paediatric Serum Application

** Test Name ‘CALA’ to link with Paediatric Serum Application ‘CALAP’

REVISION HISTORY

Added new languages

Preceding version revision history

Removed reference to obsolete calibrator.

REFERENCES

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8. Lin J, Idee JM, Port M, Diai A, Berthommier C, Robert M, et al. Interference of magnetic resonance imaging contrast agents with the serum calcium measurement technique using colorimetric reagents. J Pharm Biomed Analysis 1999;21:931-943.

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