BECKMAN COULTER

AU/DxC AU US

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.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of calcium concentrations in human serum, plasma and urine on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms). Although more than 99% of body calcium exists in bones and teeth, it is the calcium in blood which is of most concern clinically. The bones serve as a reservoir to maintain relative constancy of serum calcium by releasing calcium when required to prevent hypocalcemia and trapping calcium to prevent excessively high levels of serum calcium. The uptake and release of calcium from bone is under the control of parathyroid hormone.

The percentage of ingested calcium absorbed decreases as the dietary calcium content increases, and so the amount absorbed can remain relatively constant. The slight increase in absorption that occurs on a high-calcium diet is reflected in an increased renal excretion. Serum calcium exists in three forms: 1) free calcium ion, Ca^{2+}, 50%, 2) protein bound calcium, 45% and 3) complexed calcium, mainly with citrate, 5%. The ionized calcium is physiologically most significant but has proven difficult to assay directly. It may be estimated from total calcium given a knowledge of the protein content and pH of the blood which strongly affect the level of ionized calcium. Levels of calcium are roughly inversely proportional to phosphorus levels.

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.[1]

In disease, calcium concentration may be either higher or lower than normal. Normal levels are highest in children and decline gradually throughout life. Variations in serum calcium may be due to disease of the parathyroid gland, bone disease, defective absorption of calcium from the intestine, kidney disease, multiple myeloma and various other abnormalities.

METHODOLOGY

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]-bisbenzenear-sonic acid) to form an intense purple colored complex.[2,3]

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

Ca^{2+} + Arsenazo III $\xrightarrow[\text{Acidic Medium}]{}$ Ca-Arsenazo III complex (purple)

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Serum calcium is stable for up to 7 days at room temperature (15 – 25°C), approximately 22 days under refrigeration (2 – 8°C) and up to 1 year frozen (≤ -20°C). Urine calcium is stable for 5 days at room temperature 15 – 25°C, 5 weeks refrigerated 2 – 8°C, and 6 months frozen ≤ -20°C.[4]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum or Li/Na heparinized plasma, free from hemolysis, is the recommended specimen.

Serum or plasma should be separated from red cells as soon as possible.

Timed 24 hour urine specimens are recommended. Random collections may be appropriate if the laboratory has established its own performance characteristics.

Prior to analysis, acidify the urine specimen to a pH < 2 with 6 N HCl. Follow laboratory specific procedures for urine acidification to ensure an appropriate volume of acid is used and to avoid spurious values resulting from dilution of the sample by the acid. Samples with urine pH below 1.5 may result in a negative bias.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Calcium Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. For toxicology reporting purposes the maximum elemental arsenic concentration in wastewater would be 71.5 µg/L and organic Arsenic 370.7 µg/L of Arsenazo III. Data on file at BCI.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Imidazole (pH 6.9)	
Preservative	0.09%
Arsenazo III	0.02%

⚠ 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 (Part A)	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%
Calcium Arsenazo (Part B)	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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

Liquid Urine Chemistry Calibrator (Cat # DR0090)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR66117 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Calcium Arsenazo reagents are ready for use. No preparation is required. This reagent may be used as a Stat Calcium reagent.

STORAGE AND STABILITY

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents (routine) are stable for 90 days when stored in the refrigerated compartment of the analyzer.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, precipitate, or any change in color in the Calcium (Arsenazo) reagent may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration is once in 30 days. Calibration of this procedure is accomplished by use of the Chemistry Calibrator material (Cat # DR0070). For traceability information refer to the calibrator instructions for use.

For urine specimens use Urine Calibrator (Cat # DR0090).

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (mmol/L) the result is multiplied by 0.25.

REPORTING RESULTS

EXPECTED RESULTS

Serum[5]	8.6 – 10.3 mg/dL
Urine[5]	100 – 300 mg/day

Reference Intervals shown above were taken from the literature. 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.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

DO NOT use the following anticoagulants in collecting blood for use in this test: EDTA, Sodium Citrate, Sodium Fluoride or Oxalate.

Results of laboratory studies[6] show that the following substances interfere with this calcium determination.

The criteria for no significant interference is recovery within 10% of the initial value

Bilirubin	No significant interference up to 40 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[7]

Laboratory specific procedure notes:

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.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Calcium procedure is linear from 4.0 to 18.0 mg/dL for serum determinations and 0.1 to 40.0 mg/dL for urine determinations. Samples exceeding the upper limit of linearity should be diluted and repeated per laboratory protocol. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN, utilizing deionized water as the diluent.

Note: Care should be taken when interpreting calcium results from patients who have received gadolinium containing contrast media within the previous 24 hours especially if the patient has impaired renal function.[8,9,10,11] 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.

SENSITIVITY

The lowest detectable level using urine settings on a DxC AU analyzer was calculated as 0.07 mg/dL

The lowest detectable level using serum settings on a DxC AU analyzer was calculated as 0.25 mg/dL.

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

Reference[12]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	0.977
Intercept	0.3412
Correlation Coeff. (r)	0.9991
No. of Samples (n)	118
Range (mg/dL)	4.639 - 17.428

Patient Urine samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	1.013
Intercept	0.003
Correlation Coeff. (r)	0.9998
No. of Samples (n)	106
Range (mg/dL)	0.43 - 38.31

PRECISION

Reference[12]

Estimates of precision, based on CLSI recommendations,[13] are consistent with typical performance. The within run precision for serum samples is less than 3% CV and total precision is less than 5% CV. Assays of control sera were performed and the data reduced following the CLSI guidelines above:

Serum

N=80	**Within-run**		**Total**	
Mean mg/dL	**SD**	**CV%**	**SD**	**CV%**
9.31	0.04	0.47	0.06	0.68
12.20	0.08	0.61	0.11	0.87
15.93	0.11	0.71	0.24	1.51

Urine

N=80	**Within-run**		**Total**	
Mean mg/dL	**SD**	**CV%**	**SD**	**CV%**
2.67	0.05	1.7	0.06	2.2
24.46	0.14	0.6	0.18	0.7
38.95	0.22	0.6	0.28	0.7

Limit of Quantitation:

The Limit of Quantitation (LOQ) using serum settings for the Calcium (Arsenazo) reagent was determined to be 4 mg/dL. This was determined according to CLSI protocol EP17-A[14] and represents the lowest concentration of calcium that can be measured with a total imprecision of 20%.

ADDITIONAL INFORMATION

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

Test Name	Description
CAZ1U	Calcium Arsenazo (Serum)
CAZ1U	Calcium Arsenazo (Urine)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

Serum: † Beckman Coulter System Calibrator Cat. No.: DR0070, Point 1 – Level 1, Point 2 – Level 2

Urine: † Beckman Coulter System Calibrator Cat No.: DR0090

* Values set for working in mg/dL. To work in SI units (mmol/L) multiply by 0.25

REVISION HISTORY

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

Preceding version revision history

Add DxC 500i instrument to IFU

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Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
+(1) 800-854-3633
www.beckmancoulter.com