CALCIUM (ARSENAZO)

OSR60117	**4 x 15 mL**	**R1**
OSR61117	**4 x 29 mL**	**R1**
OSR65117*	**4 x 95 mL**	**R1**

Intended Use
System reagent for the quantitative determination of calcium concentrations in human serum, plasma and urine on Beckman Coulter AU analyzers.
*Calcium (Arsenazo) reagent OSR65117 can not be used on the AU400/AU480.

Summary
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)

System Information
For AU400/400^{e}/480, AU600/640/640^{e}/680 and AU2700/5400 Beckman Coulter Analyzers**.**

Reagents
Final concentration of reactive ingredients:

Imidazole (pH 6.9)	0.1 – 0.2%
Preservative	0.09%

Precautions
1. For in vitro diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative, which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.
4. Safety data sheet available for professional user on request.
5. Dispose of all waste material in accordance with local guidelines.
6. 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 OAI.

Preparation of Reagents
The listed 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.

Specimen Collection And Preparation
Serum or heparinized plasma, free from hemolysis, is the recommended specimen.

Calcium (Arsenazo)

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

Urine should be collected over a 24 hour period. 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.

Sample 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]

Interfering Substances
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[5] 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 1000 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. For further information on interfering substances, refer to Young[6] for a compilation of reported interference with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Calcium Reagent

Materials Required But Not Provided
Chemistry Calibrator (Cat # DR0070)
Liquid Urine Chemistry Calibrator (Cat # DR0090)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is once in 30 days. Calibration of this procedure is accomplished by use of the Chemistry Calibrator material (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 909b.
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 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 User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Appropriate qualified urine controls, should be established and utilized during urine analysis.

Results
Results are automatically printed out for each sample in mg/dL at 37°C. For SI units (mmol/L) the results must be multiplied by 0.25.

Dynamic 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.[7,8,9,10] 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.

Expected Values
Serum[11]	8.6 – 10.3 mg/dL
Urine[11]	100 – 300 mg/day

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained according to established procedures. Results obtained in individual laboratories may differ.

Precision[13]
Estimates of precision, based on CLSI recommendations,[12] 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%
8.12	0.04	0.54	0.12	1.34
12.48	0.04	0.46	0.08	0.68
13.92	0.08	0.55	0.12	0.84

Urine

N = 80	Within Run		Total	
Mean mg/dL	SD	CV%	SD	CV%
0.45	0.01	2.14	0.02	3.67
22.72	0.14	0.61	0.27	1.18
38.12	0.24	0.62	0.47	1.23

Method Comparison[13]

Serum

Patient samples were used to compare this Calcium Arsenazo Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 1
Slope	1.003
Intercept	-0.068
Correlation Coeff. (r)	0.999
No. of Samples (n)	107
Range (mg/dL)	4.05 – 15.28

Urine

Urine samples were used to compare this Calcium Arsenazo Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 1
Slope	1.020
Intercept	-0.066
Correlation Coeff.(r)	0.999
No. of Samples (n)	118
Range (mg/dL)	0.31 – 39.11

Analytical Sensitivity (Lower Detection Limit)

The lowest detectable level using serum settings on an AU analyzer was calculated as 0.13 mg/dL.
The lowest detectable level using urine settings on an AU analyzer was calculated as 0.07 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.

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%.

References

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