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Pointe Calcium Arsenazo III Reagent Set

Intended Use
Pointe Calcium (Arsenazo) Reagent Set is intended for research use only when performing the quantitative determination of Calcium in urine using the Yumizen C230 and Yumizen C240 analyzers.

Clinical Significance [1]
Urine calcium can be useful to determine osteoclastic bone reabsorption. This data helps in assessing renal stone disease and high turnover osteoporosis.

Method History
Various methodologies have been developed for the determination of calcium including flame photometry, fluorescent, gravimetric and titrimetric procedures, ion selective electrodes, and atomic absorption. Atomic absorption has been recommended as the reference method but it requires expensive instrumentation.[2]
Specific dye binding methodologies have become popular for calcium determination because they are rapid, convenient and inexpensive. Procedures using the dyes alizarin 3-sulfonate and methylthymol blue have been described.[3,4]
A method using o-cresolphthalein complexone as the chromagen was developed in 1966 by Connerty and Biggs, and modified by Gitelman in 1967 and Baginski, et al, in 1973.[5,6,7] o-Cresolphthalein complexone procedures have been widely used for the determination of calcium.
The present procedure uses Arsenazo III and has been modified to provide a highly sensitive and stable reagent system. Magnesium interference is prevented by the inclusion of 8-hydroxyquinoline sulfonate. The reagent is provided as a convenient ready to use liquid.

Principle
Alkaline
Calcium + Arsenazo ----------➢ Calcium-Arsenazo Complex
Medium (purple color)

Calcium reacts with Arsenazo III in a slightly alkaline medium to form a purple-colored complex which absorbs at 650 nm. The intensity of the color is proportional to the calcium concentration.

Reagents
Calcium reagent: Arsenazo III ≥ 0.15mM, 8-Hyrdroxyquinoline Sulfonate 5.0mM, Buffer, Surfactant.

Reagent Preparation
Reagent is ready to use.

Reagent Storage
Store reagent at room temperature (15-30°C). The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use if the reagent has become noticeably turbid.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagent may be irritating to the skin. Avoid contact. Flush with water if contact occurs.

Specimen Collection and Storage
1. Fresh, urine is the preferred specimen.
2. 24 hours urine specimens must be collected with HCl 6N. Non acidified urines which have been refrigerated should be acidified and/or heated and allowed to stand for 1hr before remixing and aliquoting.[1]
3. Urine calcium is stable for 2 days at room temperature, 4 days at 2-8°C, and up to 3 weeks at -20°C when acidified to pH<2.[8]

Interferences
1. Alkalinized urine can cause falsely decreased measurements of urine calcium due to precipitation of salts. Acidification of urine is recommended to improve accuracy. [1]
2. For a comprehensive review of interferences see Young, et al.[9]

Materials Provided
Calcium Reagent

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Human Urine control set, catalog number P7582-CTL

Test Parameters

Chem:	CA	Chemistry:	Calcium (Arsenazo)
Chemistry No.:	209	Print Name:	CA
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	670 nm	Sec. Wave	
Decimal.:	0.1	Samp. Type:	Urine
Blank Time:		Reaction Time:	7 8
Unit:	mg/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	3 uL	uL	uL	R1: 250 uL	uL
Decreased;		uL	uL	uL	
Increased;		uL	uL	uL	

Linearity Range (Standard);	0.0-18.0		Linearity Limit:	
Linearity Range (Decreased):			Substrate Depletion:	
Linearity Range (Increased):			Mixed Blank Abs.:	- 40000 40000
R1 Blank Abs.:	- 40000	40000	On-board Stability:	30 Day (s)
Blank Response	- 40000	40000	Reagent Alarm Limit:	10
Twin Chemistry:				

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	mg/dL

Pretreatment:			
Pretreat Sample Vol.:	ulL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Calibration Setup Parameters

Chem:	CA		
Calibration Setting			
Math Model:	Two-Point Linear		
Factor:	Replicates: 2		
Acceptance Limits			
Cal Time:	hr.		
Slope Diff:	SD:		
Sensitivity:	Repeatability:		
Deter Coeff:			
Auto Calib.			
□ Cal Time			

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

* User Defined

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Limitations
The linearity limit for the calcium in samples is 18mg/dl

Calibration
Use an NIST-traceable calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculations (Example)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Std.} = \text{Calcium (mg/dl)}$$

Example: If the absorbance of sample = 0.81, absorbance of standard = 0.80, concentration of standard = 10mg/dl, then:

$$\frac{0.81}{0.80} \times 10 = 10.1\text{mg/dl}$$

NOTE: To correct mg/dl to mEq/L, divide mg/dl value by two.

Quality Control
The integrity of the reaction should be monitored by use of normal and abnormal control samples with known calcium concentrations. These controls should be run at least with every working shift in which calcium assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Value
10-20 grams/day[1]
It is strongly recommended that each laboratory establish its own reference range.

Performance
1. Linearity: 0.0-18.0 mg/dl
2. Comparison: A study was performed between the Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of 0.9969 with a regression equation of y=0.933x + 0.653 (n=43).
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

	Within Run	
Mean	**S.D.**	**C.V.%**
6.1	0.27	4.5
8.4	0.05	0.6
12.1	0.09	0.7

4. Sensitivity: The limit of blank (LOB): 0.2mg/dl.

References

1. Nader Rifai, Andrea Rita Horvath, Carl T. Wittwer. Tietz textbook of clinical chemistry and molecular diagnostics. Sixth Edition. St. Louis : Elsevier, 2018. P.1438-1439
2. Cali, J.P., et al, N.B.S., Sp. Publication 260:36 (1972).
3. Connerty, H.V. and Biggs, A.r., Am. J. Clin. Chem. 11:716 (1965).
4. Gindler, E.M. and King, J.D., Am. J. Clin. Path. 58:376 (1972).
5. Connerty, H.V. and Biggs, A.R., Am. J. Clin. Path. 45:290 (1966).
6. Gitelman, H.J., Anal. Biochem. 18:521 (1967).
7. Baginski, E.S., et al, Clin. Chem. Acta 46:49 (1973).
8. Use of anticoagulants in diagnostic laboratory investigations. WHO publication WHO/DIL/LAB/99.1Rev.2, p.46 (2002).
9. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1995) 3: 122-124.
10. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use

Research use only

REF 12-C7529-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
5449 Research Drive Canton, MI 48188

Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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