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Pointe Calcium Arsenazo III Reagent Set

Intended Use
For the quantitative determination of Calcium in serum or heparinized plasma using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

Clinical Significance [1,2]
Increased serum calcium may be observed in hyperparathyroidism, vitamin D intoxication, multiple myeloma and some neoplastic diseases of bone. Decreased serum calcium may be observed in hypoparathyroidism, vitamin D deficiency, steatorrhea, nephrosis, and nephritis.

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.[3]
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.[4,5]
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.[6,7,8] 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
$$\text{Calcium} + \text{Arsenazo} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Calcium-Arsenazo Complex (purple color)}$$

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, unhemolyzed serum is the preferred specimen.
2. Heparinized plasma may also be used.
3. Anticoagulants other than heparin should not be used.[9]
4. Remove serum from clot as soon as possible since red cells can absorb calcium.[10]
5. Older serum specimens containing visible precipitate should not be used.[11,12]
6. Serum calcium is stable for 24 hours at room temperature, one week at 2-8°C, and up to five months frozen (-15 to -25°C) and protected from evaporation.[13] Specimens should not be thawed and refrozen.

Interferences
1. Substances that contain or complex with calcium cause inaccurate results.[14]
2. Glass tubes often are coated with a residue containing calcium. They should be acid-washed before use. Alternatively, plastic tubes may be used.
3. Bilirubin up to 20 mg/dl and hemoglobin to 500 mg/dl do not interfere.
4. Severe lipemia may cause elevated results. A serum blank should be run for greatest accuracy.
5. For a comprehensive review of interferences see Young, et al.[15]

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. Chemistry control, catalog number C7592-100

Test Parameters

Test:	CA	Chemistry:	Calcium
Chemistry No.:	209	Print Name:	CA
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	670 nm	Sec. Wave	
Decimal.:	0.1	Samp. Type:	Serum
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);	0-15		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:	5		
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:

Pointe
Calcium Arsenazo III
Reagent Set

Calibration Setup Parameters

Chem: CA
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
□ Cal Time

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

Limitations

Samples with calcium values exceeding 15mg/dl[16] should be diluted with an equal volume of saline, the assay repeated, and the result multiplied by two. Severely lipemic samples should be run with a serum blank for greatest accuracy.

Calibration

Use an NIST-traceable serum 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 sera 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

Adults: 8.5-10.4 mg/dl[17]
Newborns: 7.8-11.2 mg/dl[18]

It is strongly recommended that each laboratory establish its own reference range.

Performance

1. Linearity: 0-15 mg/dl[16]
2. Comparison: A study was performed between the Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of 0.994 with a regression equation of y=0.99x + 0.18 (n=36).
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.[19]

Within Run			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
9.91	0.48	4.8	8.96	0.19	2.12
12.12	0.57	4.7	11.14	0.26	2.33

4. Sensitivity: Recovery studies indicate that this reagent can distinguish calcium concentrations of 0.1 mg/dl throughout the linear range of the assay.[16]

References

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5. Gindler, E.M. and King, J.D., Am. J. Clin. Path. 58:376 (1972).
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12. Tayeau, F., et al, Bull. Soc. Pharm. Bordeaux, 95:206 (1956).
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16. HORIBA Medical records.
17. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p. 1208 (1984).
18. Meites, Samuel, Pediatric Clinical Chemistry, Washington DC, AACC Press, p. 81 (1989).
19. 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
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-C7529-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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CE

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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