Pointe Calcium (Arsenazo) Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of Calcium in serum or heparinized plasma using the Yumizen C560 analyzer. **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 and Stability
Store reagent at room temperature (15-30°C). The reagent is stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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

Precautions and Hazards
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.

Hazards:
Hazard Classifications: Reproductive Toxicity (Category 2)
Hazard Statements: H361: Suspected of damaging fertility or the unborn child
Precautionary Statements: **Prevention:** P202 Do not handle until all safety precautions have been read and understood. P280 Wear protective gloves/protective clothing/eye protection/face protection. **Response:** P308 + P313 IF exposed or concerned: Get medical advice/attention. **Storage:** P404 Store in a closed container. **Disposal:** P501: Dispose of contents to an approved waste disposal plant. **Refer to the Safety Data Sheet for this product (SDS-C7529) available by calling 1-734-487-8300.**

Signal Word: Warning

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]

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Materials Provided
Calcium Reagent

Materials Required but not Provided
1. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

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 test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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. Assay Range: 0.1-15 mg/dl[16]
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	Calcium
N	139
Mean Calcium (mg/dL)	9.32
Range (mg/dL)	0.6-14.5
Standard Deviation	3.89
Regression Analysis	y = 1.093x – 1.02
Correlation Coefficient	0.9808

3. Precision: Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[19]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	5.04	9.32	12.98
Standard Deviation	0.07	0.13	0.16
Coefficient of Variation (%)	1.3%	1.4%	1.2%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	5.21	9.35	12.95
Standard Deviation	0.17	0.15	0.24
Coefficient of Variation (%)	3.3%	1.6%	1.9%

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.1 mg/dL

References

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CHEMISTRY PARAMETERS

Chem:	CA	No.:	209	Sample Type:	Serum
Chemistry:	Calcium (Arsenazo)			Print Name:	CA
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	660			Sec Wave:	
Unit:	mg/dL			Decimal	0.1
Blank Time:	10 12			Reaction Time:	27 29

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	1.5 ul	--- ul	--- ul	R1: 125 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0.1	15	Linearity Limit:	
Linearity Range (Decreased)	____	____	Substrate Depletion:	
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	CA	0	mg/dL
Chemistry Calibrator	*	*	*	CA	*	mg/dL

Calibration Setup

Chem:	CA		
Calibration Settings			
Math Model:	Two-Point Linear		
Factor:		Replicates:	2
Acceptance Limits			
Cal Time:	336	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-C7529-360

Manufactured by
HORIBA Instruments Incorporated-Pointe Brand
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IVD

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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Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only