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Calcium Arsenazo III Reagent Set

Intended Use
For the quantitative determination of Calcium in serum or heparinized plasma.

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-Hydroxyquinoline Sulfonate 5.0mM, Buffer, Surfactant.

Reagent Preparation
Reagent is ready to use.

Reagent Storage
Store reagent at room temperature (15-30°C).

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. (See Procedure Notes.)
5. For a comprehensive review of interferences see Young, et al.[15]

Materials Provided
Calcium Reagent.

Materials Required but not Provided
1. Accurate pipetting devices.
2. Timer.
3. Acid-washed glass or plastic test tubes and rack.
4. Spectrophotometer able to read at 650 nm (600-670nm).

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Label test tubes “reagent blank”, “standard”, “control”, “sample”, etc.
2. Pipette 1.0 ml of reagent into each tube.
3. Add 0.010 ml (10ul) of sample to respective tubes. Mix and let stand at room temperature for at least one minute.
4. Zero spectrophotometer with the reagent blank at 650 nm.
5. Read and record absorbances of all test tubes.
6. To calculate results see “Calculations”.

Procedure Notes
1. Final color is stable for 60 minutes.
2. Samples with calcium above 15 mg/dl should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
3. Severely lipemic samples require a serum blank. Add 10ul of sample to 1.0ml distilled water. Read against water at 650nm and subtract the absorbance reading from the test absorbance.
4. Contamination of glassware with calcium will adversely affect test results. Acid-washed glass or plastic test tubes are recommended.
5. Reagent and sample volumes may need to be adjusted according to the requirements of the specific spectrophotometer being used.

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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. See "Procedure Notes".

Calibration

Use an aqueous Calcium Standard (10mg/dl) or an appropriate serum calibrator. The material used for calibration should be traceable to NIST material.

Calculations

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

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 their own frequency of control determination.

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 performed with an o-cresolphthalein complexone procedure yielded a correlation coefficient of 0.989 with a regression equation of y=0.99x + 0.10 (n=80, range=4.7-15.9 mg/dl).
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
11.0	0.14	1.3	11.2	0.12	1.1
14.3	0.13	0.9	14.3	0.18	1.3

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]

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