Calcium Reagent
Catalog #: 80133
for use with the LIASYS-330 CLINICAL CHEMISTRY SYSTEM

INTENDED USE
For the in vitro quantitative determination of Calcium in serum.

CLINICAL SIGNIFICANCE
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.

METHODOLOGY
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.

Specific dye binding methodologies have become popular for calcium determination because they are rapid, convenient and inexpensive. Procedures using the dyes alizarin3-sulfonate and methylthymol blue have been described. A method using o-Cresolphthalein Complexone as the chromagen was developed in 1966 by Connelly and Biggs, and modified by Gitelman in 1967 and Baginski et al in 1973. 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. The reagent is provided as a convenient ready to use liquid.

Principle
Calcium + Arsenazo III → Calcium-Arsenazo Complex

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.

REAGENT COMPOSITION
Active Ingredients
Arsenazo III
Imidazol Buffer
Surfactant
pH 6.75 ± 0.2

Concentration
0.2 mM
100 mM

REAGENT PREPARATION
Reagent is supplied ready to use.

STABILITY AND STORAGE
When stored at 2-25°C, the reagent is stable for the expiration date stated on the label.

REAGENT DETERIORATION
The reagent should not be used if:
1. The reagent is turbid.
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND STORAGE
1. Fresh, unhemolyzed serum is the preferred specimen.
2. Anticoagulants other than heparin should not be used.
3. Remove serum from clot as soon as possible since red cells can absorb calcium.
4. Older serum specimens containing visible precipitate should not be used.
5. Serum calcium is stable for one week at refrigerated (2-6°C), and up to five months frozen (-15 to -25°C) when protected from evaporation. Specimens should not be thawed and then refrozen.

INTERFERENCES
Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:
No significant interference (±10%) from hemoglobin up to 400 mg/dL.

Bilirubin:
No significant interference (±10%) from bilirubin up to 34.7 mg/dL.

Lipemia:
No significant interference (±10%) from lipemia up to 31.0 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
1. LIASYS 330 analyzer.
2. Deionized water and related equipment, e.g.: pipettes.
3. Analyzer specific consumables, e.g.: sample cups.

ASSAY PROCEDURE

Calcium

TEMPERATURE: 37°C
WAVELENGTH: 650 nm

ASSAY TYPE: Endpoint
DIRECTION: Increase

SAMPLE / RGT RATIO: 1 : 50

e.g. Sample Vol 0.02 mL (20mL)

Diluted Sample

INCUBATION: ³ 1 min

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 may require a serum blank.
4. Contamination of glassware with calcium will adversely affect test results. Acid-washed glass or plastic test tubes are recommended.

Calculation

Absorbance of sample = Conc. of Calcium (mg/dL) x
Absorbance of standard = Std.

Example:

Absorbance of sample = 0.81
Absorbance of standard = 0.80
Concentration of standard = 10 mg/dL

0.81 x 10 = 10.1 mg/dL Calcium
0.80

LIMITATIONS
1. Samples with calcium values exceeding 15 mg/dL should be diluted with an equal volume of distilled water, the assay repeated, and the result multiplied by two.
2. Severely lipemic samples should be run with a serum blank for greatest accuracy.

QUALITY CONTROL
Use an aqueous Calcium standard or an appropriate serum calibrator.

EXPECTED VALUES
Adults: 8.5 - 10.4 mg/dL
Newborns: 7.8 - 11.2 mg/dL

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

REFERENCE