CALCIUM

(LIQUID)

4 x 60 ml

RE – ORDER CAL1080

INTENDED USE:

This reagent is intended for the quantitative in vitro measurement of the total calcium concentration in serum or heparinized plasma.

TEST SUMMARY AND EXPLANATION:

Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).

Accurate and precise measurement of calcium in biological fluids has traditionally been difficult. Atomic absorption spectro-photometry (AAS) and the spectrophotometric measurement of calcium-dye complexes such as o-cresolphthalein complexone (CPC) and Arsenazo III are most often used for measuring total calcium (1). Variations of the latter method have been applied to automated analyzers.

TEST PRINCIPLE:

The Raichem method for the determination of calcium presented here uses Arsenazo III (3,6-bis [(2-Arsonophenyl) Azo] -4,5-di-hydroxy-2, 7-naphthalenedisulfonic acid) (2); CAS registry number: 1668-00-4.

Arsenazo III is chemically stable and has a very high affinity for calcium in a neutral pH range. In this assay system, the Arsenazo III forms a 1:1 violet Arsenazo III: calcium complex with an absorbance maximum at 650 nm (3). The concentration of calcium is proportional to the absorbance of the violet colored Arsenazo III: calcium complex. The color produced by this complex is stable for at least 8 hours at room temperature (22–28 °C).

Magnesium does not interfere in this assay system. The affinity of Arsenazo III for magnesium is essentially zero at the pH at which the assay is performed (2).

This single reagent is provided in a stable, ready-to-use liquid form.

REAGENT COMPOSITION:

The reagent solution has the following approximate concentrations of components:

Reactive ingredients:

Arsenazo III	233 µmol/L

Non-reactive buffers, stabilizers and fillers

REAGENT PREPARATION:

The reagent provided is ready for use.

REAGENT STORAGE AND STABILITY:

The reagent is stable at room temperature (22–28 °C) until the expiration date on the label. If the absorbance of the reagent alone (without sample added) in a 1 cm cuvette is greater than 0.500 when measured against water at 650 nm, do not use the reagent.

PRECAUTIONS:

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION AND STORAGE:

Serum is the preferred specimen. Heparinized plasma may also be used (1). Hemolyzed samples should not be used.

Plasma prepared using EDTA, oxalate, citrate, which function by removal of calcium, obviously must not be used.

Store serum samples in the refrigerator (2–8 °C).

INTERFERING SUBSTANCES:

Any substance which either chelates calcium or contains calcium will interfere with the assay. When magnesium concentrations in incremental amounts of 1, 2, 4 and 6 mg/dL (magnesium expected range value: 1.8 to 2.9 mg/dL) were added to 12 serum samples with calcium values ranging from 7.5 to 9.9 mg/dL, there was no increase in the calcium values. There was also no increase due to magnesium in the calcium value (9.5 ± 0.8 mg/dL) in 12 assays of a control serum to which magnesium was added in concentrations ranging from 0.5 to 15 mg/dL (4). In very rare cases a monoclonal gammopathy may lead to falsely elevated calcium values due to formation of turbidity during the assay procedure or direct interaction of the monoclonal antibody in the specimen with the test system. Gammopathy samples must be evaluated by another Calcium method. Young et al. (5) have published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that for serum calcium. Interference from lipemia is minimized because of the small amount of sample used, however, >10% elevation in Calcium results may occur for samples with moderate to gross turbidity.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 650 nm.

Matched cuvettes.

Distilled or deionized water.

Pipettes to measure water, reagent, samples and standard.

Standard or calibrator with an established value for calcium concentration.

TEST PROCEDURE:

Wavelength: 650 nm

Into a series of matched cuvettes pipette:

Test:	1 mL reagent + 25 µL sample
Standard:	1 mL reagent + 25 µL standard
Blank:	1 mL reagent + 25 µL water

Mix. Incubate at room temperature. The reaction is complete within one minute. The color is stable for at least 8 hours.

Read the absorbance of the sample and standard at 650 nm, adjusting the instrument to zero absorbance with the blank.

CALIBRATION:

This assay requires the use of a calcium standard. Use commercially available standards or calibrators.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for calcium concentration may be used. Commercially Assayed Control Serum, Level 1 and Level 2 are recommended for this purpose.

CALCULATIONS:

$$\frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times \text{Conc. of std.} = \text{mg/dL calcium in sample.}$$

Sample Calculation:

If the absorbance of the sample is 0.581 and that of the standard is 0.645 and the concentration of the standard is 10 mg/dL:

$$\frac{0.581}{0.645} \times 10 = 9.0 \text{ mg/dL}$$

IIMITATIONS OF THE PROCEDURE:

Samples with calcium concentrations exceeding 15 mg/dL should be diluted with an equal volume of distilled or deionized water and the assay repeated; multiply results by 2.

REAGENT PERFORMANCE:

Performance studies were performed using serum samples.

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Linearity: The assay is linear to 15 mg/dL calcium.

Correlation: Employing the Calcium o-Cresolph-thalein (Catalogue Nos. 84104 and 84105) as a reference method, 72 serum samples ranging from 6.0 mg/dL to 18.0 mg/dL were assayed. The correlation coefficient was 0.998 and the regression equation was y = 0.99984x + 0.043.

A comparison of this reagent was made using as a reference the method of Clark and Collip by titration of the precipitated calcium oxalate with potassium permanganate. Twenty serum samples ranging from 7.4 mg/dL to 10.6 mg/dL calcium were assayed. The correlation coefficient was 0.997 and the regression equation was y = 1.05x - 0.52.

Precision:

Within Run			
Mean	6.8	12.9	21.8
SD	0.005	0.117	0.13
CV	0.07%	0.9%	0.7%
N	9	9	9
Run to Run			
Mean	6.8	12.9	21.8
SD	0.08	0.10	0.18
CV	1.2%	0.7%	0.8%
N	27	27	27

Sensitivity: Using a 1:40 sample to reagent ratio and reading at 650 nm, a 1 mg/dL calcium sample will produce a net absorbance of approximately 0.065.

REFERENCE RANGE:

The following reference range is from references (7, 8, 9):

Adaults:8.5 to 10.4 mg/dL (2.1 to 2.6 mmol/L).

Newborns: 7.8-11.2

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

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