MEDICA

IVD CE

REF 10210-4 4 x 29 mL / 9 mL

TOTAL CALCIUM (CA)

Wedges each contain usable volumes of 29 mL of R1 reagent and 9 mL of R2 acid cleaning solution.

INTENDED USE

The EasyRA® CA reagent is intended for the quantitative measurement of total calcium in human serum or plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA Clinical Chemistry Analyzer."
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Total calcium in serum is composed of free calcium, calcium complexed to anions, and calcium bound to proteins (primarily albumin). Calcium plays a key role in many intracellular (muscle contraction, glycogen metabolism) and extracellular (bone mineralization) processes. 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).

PRINCIPLE OF THE PROCEDURE

The total calcium reagent utilizes Arsenazo III, which is very stable and has a high affinity for calcium at neutral pH. Interference from magnesium is eliminated by the addition of 8-hydroxyquinoline-5-sulfonic acid.[1]

Ca^{++} + Arsenazo III $\rightarrow$ colored complex (blue)

Arsenazo III reacts with calcium to form a 1:1 blue complex with an absorption maximum at 650 nm. The intensity of the blue color is directly proportional to the concentration of Total Calcium in the sample.

REAGENTS

Calcium Reagent (R1):

Phosphate buffer, pH 7.5	50 mmol/L
8-Hydroxyquinoline-5-sulfonic acid	5 mmol/L
Arsenazo III	0.12 mmol/L
non-reactive stabilizers and surfactant	

The R2 segment of the wedge contains acid to clean the probe prior to performing the Calcium test:

Hydrochloric Acid	50mmol/L
Surfactant	

Precautions

1. Organic arsenic compounds have been classified as potentially carcinogenic, therefore, safe laboratory practices should be carefully observed.
2. Good laboratory safety practices should be followed when handling any laboratory reagent (CLSI, GP17-A2).
3. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
4. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
5. A major source of error with this assay is calcium contamination. Many detergents and water supplies contain calcium. Incompletely rinsed cuvettes used in the test will lead to inaccurate results. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. Do not use the reagent if it is turbid or cloudy.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum or plasma should be used. Lithium Heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum as soon as possible after collection. Serum Calcium is stable for 3 weeks at 2 – 8°C.

PROCEDURE

Materials Provided

Medica CA Reagent Wedge, REF 10210

Additional materials required

Medica EasyCal Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use

The reagent is ready to use as supplied. Place the reagent in the EasyRA analyzer reagent tray located in the reagent area. When used this way, the reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge (21 days maximum).

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (14 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a new wedge placed on the analyzer, a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA Analyzer calculates the calcium concentration from the ratio of the absorbance of the sample to the absorbance of the calibrator. Values are derived based on the blue complex color intensity read at 600 nm and blanked at 700 nm.

$$\text{Calcium (mg/dL)} = \frac{[(A_U - A_{Blk})_{600} - (A_U - A_{Blk})_{700}]}{[(A_C - A_{Blk})_{600} - (A_C - A_{Blk})_{700}]} \times \text{Cal Value}$$

Where A_U and A_C are the absorbance values of the unknown and the calibrator, respectively; A_{Blk} is the absorbance of the reagent blank; and "Cal Value" is the concentration of Calcium in the calibrator (mg/dL).

Expected Values[2]

The reference range for Calcium in serum is as follows:

Normal: 8.8-10.2 mg/dL

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g., if sample is above assay range)

Avoid using heavily hemolyzed serum or plasma samples.

The EasyRA Analyzer flags any result above 15 mg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will effectively extend the reportable range of the CA test to 30 mg/dL.

PERFORMANCE CHARACTERISTICS[3]

Reportable Range

The reportable range is 1 to 15 mg/dL. Extended range is 1 to 30 mg/dL when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)

The following table lists the data obtained in a comparison of the Medica reagent for Calcium (y) on the EasyRA Analyzer to the performance of a similar Calcium reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations on the EasyRA Analyzer vs. the average of two replicate values obtained on the COBAS MIRA Analyzer.

Number of Samples	49	Range of Samples	1.7 to 13.2 mg/dL
Slope	1.06	y Intercept	-0.13
Correlation Coefficient	0.9874	Regression Equation	Y = 1.06*X - 0.13

**Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

The following table lists the data obtained in a comparison of matched serum (x) and Li-heparinized plasma (y) samples using the Medica reagent for CA on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	70	Range of Samples	1.62 to 14.71 mg/dL
Slope	0.9854	y Intercept	-0.0643
Correlation	0.9891	Regression Equation	Y = 0.9854*X – 0.0643

Imprecision (CLSI, EP5-A2)
Within run imprecision: Five replicates of each of three levels of commercial human serum-based QC material were tested per day over 5 days.

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
12.81	0.23	1.8
9.73	0.19	1.9
5.24	0.17	3.3

Total Imprecision: Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days.

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
11.78	0.16	1.33
9.03	0.13	1.46
5.95	0.12	1.95

Linearity (CLSI, EP6-A)

Linear from 1 to 15 mg/dL, based on the linear regression Y = 0.968*X – 0.089.

Interfering Substances (CLSI, EP-7A)
Less than 10% interference was classified as “no significant interference.”
No significant interference was found in levels of up to 500 mg/dL of hemoglobin.
No significant interference was found in levels up to 20 mg/dL of bilirubin.
No significant interference was found in levels of up to 2000 mg/dL of triglycerides (using Intralipid*).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[4,5]

REFERENCES

1. Morgan, BR, Artiss, JD and Zak, B. *Calcium Determination in serum with Sable Alkaline Aresazo III and Triglyceride Clearing*. Clin Chem 1993; 39: 1608-1612.
2 Tietz NW. *Textbook of Clinical Chemistry*, 3rd ed. WB Saunders and Co., Philadelphia, PA, p. 831-832 (1994).
3 Data on file at Medica.
4 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests.* 2nd ed. Washington, DC. AACC Press; 1997.
5 Young DS. *Effects of Drugs on Clinical Laboratory Tests*. 4th ed. Washington, DC: AACC Press; 1995.

EASYRA ASSAY PARAMETERS (CA)

Primary Wavelength (nm)	600
Secondary Wavelength (nm)	700
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	No
Reaction Time	2.0 min
Calibration interval (maximum)	14 days
Reagent on-board stability	21 days

Serum/ Plasma

Sample volume (µl)	4.5
Diluent volume (µl)	20
Reagent volume (µl)	350
Decimal Places (default)	2
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	1 to 15 mg/dL

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