

## DIAGNOSTIC KIT FOR DETERMINATION OF CALCIUM CONCENTRATION



### HC – CALCIUM ARSENAZO

#### INTRODUCTION

Calcium plays an essential role in many cell functions: intracellularly in muscle contraction and glycogen metabolism, extracellularly, in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium is present in plasma in three forms: free, bound to proteins or complexed with anions as phosphate, citrate and bicarbonate. Decreased total calcium levels can be associated with diseases of the bone apparatus (especially osteoporosis), kidney diseases (especially under dialysis), defective intestinal absorption and hypoparathyroidism. Increased total calcium can be measured in hyperparathyroidism, malignant diseases with metastases and sarcoidosis. Calcium measurements also help in monitoring of calcium supplementation mainly in the prevention of osteoporosis.

#### METHOD PRINCIPLE

Photometric test using arsenazo III.

Calcium with arsenazo III at neutral pH yields a blue colored complex, whose intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline-5-sulfonic acid.

#### REAGENTS

##### Package

1-Reagent 2 x 97.5 ml

The reagent when stored at 2-8°C is stable up to expiry date printed on the package. The reagents are stable for 10 weeks on board the analyser at 2-10°C. Do not freeze the reagent! Protect from light and avoid contamination!

##### Concentrations in the test

phosphate buffer (pH 7.5)	67 mmol/l
8-hydroxyquinoline-5-sulfonic acid	5 mmol/l
arsenazo III	100 µmol/l
detergents	

#### Warnings and notes

- Product for in vitro diagnostic use only.
- Contaminated glassware is the greatest source of error. The use of disposable plastic ware is recommended. Glassware should be soaked for a few hours in 2M HCl solution and then thoroughly rinsed with distilled water.

#### SPECIMEN

Serum. Random or 24-hours urine.

Serum can be stored up to 7 days at 20-25°C or up to 3 weeks at 4-8°C. Samples frozen at -20°C can be stored up to 8 months. Discard contaminated specimens.

24-hours urine preparation: To prevent calcium salt precipitation specimens should be collected in 10 ml of 6M HCl. In case of presence of precipitants they can be solved by lowering pH of the urine to below 2.0.

Nevertheless it is recommended to perform the assay with freshly collected samples!

#### PROCEDURE

The reagent is ready to use.

This reagent may be used in automatic analyser Hitachi 911/912.

Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:

1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.

4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

#### REFERENCE VALUES <sup>4</sup>

serum	mg/dl	mmol/l
adult	8.6 – 10.3	2.15 – 2.57
random urine	mg/dl	mmol/l
male	0.9 – 37.9	0.225 – 9.47
female	0.5 – 35.7	0.125 – 8.92
24-hours urine	mg/dl	mmol/l
adult	100 – 300	2.5 – 7.5

It is recommended for each laboratory to establish its own reference ranges for local population.

#### QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) is recommended. **Calibrator and deionised water** should be used for calibration.

The calibration curve should be prepared every 10 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

#### PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.28 mg/dl (0.07 mmol/l).
- **Linearity:** up to 25 mg/dl (6.25 mmol/l).  
For higher concentration dilute the sample 1+1 with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.
- **Specificity / Interferences**  
Haemoglobin up to 1.88 g/dl, ascorbate up to 62 mg/l, bilirubin up to 40 mg/dl, triglycerides up to 1000 mg/dl and magnesium up to 20 mg/dl do not interfere with the test.

##### Precision

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	8.86	0.07	0.81
level 2	11.53	0.06	0.50

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	9.33	0.10	1.07
level 2	12.01	0.16	1.35

##### Method comparison

A comparison between calcium values determined at Hitachi 912 (y) and at Advia 1650 (x) using 99 samples gave following results:

$$y = 0.9242 x + 0.4608 \text{ mg/dl};$$

$$R = 0.9721$$

(R – correlation coefficient)

## WASTE MANAGEMENT

Please refer to local legal requirements.

## LITERATURE

1. Endres DB, Rude RK. Mineral and bone metabolism. In: . Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3<sup>rd</sup> ed. Philadelphia, PA: Moss D.W., Henderson A. R. (1999) p. 1395-1457.
2. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971;53: 194-8. Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981; 110:61-72.
3. Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981; 110:61-72.
4. Alan H.B. Wu. editor. Tietz Clinical Guide to Laboratory Tests, 4<sup>th</sup> ed. St. Louis: W.B Saunders Company; 2006, p. 202-204.

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