



Intended Use

The reagents are used for the quantitative determination of Calcium in serum or plasma. For in-vitro diagnostic use only.

Introduction

Calcium is the most abundant mineral in the human body. The average adult body contains approximately 1 kg of this element, 99% of which is in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the serum. Calcium metabolism or calcium homeostasis is the mechanism by which the body maintains adequate calcium levels. Derangements of this mechanism lead to hypercalcemia or hypocalcemia, which both can have important consequences for health.

Hypercalcemia

It may be developed in case of abnormal parathyroid gland function, malignancy, vitamin-D metabolic disorders, disorders related to high bone turnover rate like Paget's disease and renal failure.

Hypocalcemia

This may be developed in case of rickets, coeliac disease, idiopathic steatorrhea, osteomalacia and following surgical resection of small intestine.

Method

Ortho-Cresolphthalein Complexone (OCPC) method of Moorehead and Briggs, End Point.

Principle

The calcium in the patient serum/plasma reacts with OCPC to form a purple colored complex. The intensity of the color is directly proportional to the concentration of calcium in the sample. The concentration is measured photometrically at a wavelength of 578nm (550 – 590nm) and compared with that of a standard.

Reagent Composition

Reagent 1:

| | |
|-----------------------------|------------|
| 2-Amino-2-methyl-1-propanol | 680 mmol/l |
|-----------------------------|------------|

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Reagent 2:

| | |
|--------------------|--------------|
| o-Cresolphthaleine | 0.157 mmol/l |
| 8-Hydroxyquinoline | 6.9 mmol/l |

Reagent 3 (Washing solution):

| | |
|-------------------|-------|
| Hydrochloric acid | 0.1 M |
|-------------------|-------|

Reagent 4:

| | |
|------------------|---------|
| Calcium Standard | 8 mg/dl |
|------------------|---------|

Precautions

Following precautions should be taken:

- All the glassware should be washed with 0.1 M HCl (Washing solution) and properly rinsed with distilled water and dried before use.
- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened Reagents (Reagent 1, 2, 3) are stable till expiry mentioned on the label when stored at room temperature (below 30°C).

Standard Reagent 4 is stable till expiry mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 5 (Calcium: 12 mg/dl) & Reagent 6 (Calcium: 15 mg/dl) can be provided for linearity check with Reagent 4 (Calcium: 8 mg/dl- Standard).

Reagent Preparation

Mix equal volume of Reagent 1 & 2 for the preparation of working solution for the required number of tests. The working solution is stable for one week when stored at 2-8°C.

Reagent Deterioration

Reagent should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Serum or heparinized plasma can be used for the testing. Other anti-coagulants like EDTA, Citrate and Oxalates are not suitable for the assay. Samples can be stored for 7 days at 2-8°C..

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General Assay Parameters

| | |
|----------------------------|-----------|
| Mode | End Point |
| Wavelength 1 (nm) | 578 |
| Blank with | Reagent |
| Sample Volume (µl) | 10/20 |
| Working Rgt.(µl) | 500/1000 |
| Incubation Time (min.) | 5 |
| Incubation Temperature(°C) | RT |
| Normal Low (mg/dl) | 8.1 |
| Normal High (mg/dl) | 10.4 |
| Linearity (mg/dl) | Upto 15 |
| Standard Conc. (mg/dl) | 8 |
| Units | mg/dl |

PROCEDURE

Always use 0.1 M HCl, washed & dried test tubes before test the assay. One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

| Particulars | Blank | Standard | Sample |
|-----------------|---------|----------|---------|
| Working Reagent | 1000 µl | 1000 µl | 1000 µl |
| Dist. Water | 20 µl | - | - |
| Reagent 4 | - | 20 µl | - |
| Sample | - | - | 20 µl |

Mix well & incubate for 5 min at room temperature. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 578nm

Calculation

Calcium concentration in the sample can be calculated using the following formula:

$$\text{Calcium} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

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$$\frac{0.200}{0.180} \times 8.0 = 8.88 \text{ mg/dl}$$

If the calcium concentration exceeds 15mg/dl, dilute the sample and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value
8.1 – 10.4 mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

Limitations

- The reagent and sample volumes can be altered proportionately so that the sample: reagent ratio remains same.
- Calibration using aqueous standard provided in the kit may cause a matrix effect in some analyzers. In such a case it is recommended to use a serum based calibrator.
- Calcium contamination should be avoided. It is recommended to use unused plastic tubes or cuvettes free from calcium. For glassware use the washing solution to avoid contamination.
- Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample blank may be used as mentioned below:
 - Add 20µl of serum sample to 1000µl of DI water and read absorbance at 578nm.
 - Subtract the absorbance obtained as above, from the absorbance of test. Use this corrected absorbance for calculation.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for calcium.

Performance

Linearity Limit: 15 mg/dl

Precision:

Within run

| Control | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 20 | 20 |
| Mean (mg/dl) | 9.059 | 12.182 |
| S.D. | 0.118 | 0.226 |
| C.V. % | 1.303 | 1.853 |

Between run

| Control | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 60 | 60 |
| Mean (mg/dl) | 9.02 | 12.12 |
| S.D. | 0.10 | 0.17 |
| C.V. % | 1.14 | 1.45 |

References

- Marshall, W. J. 1995. Clinical Chemistry, 3rd ed. Mosby, London.
- Tierney, Lawrence M.; McPhee, Stephen J.; Papadakis, Maxine A. (2006). Current Medical Diagnosis and Treatment 2007 (Current Medical Diagnosis and Treatment). McGraw-Hill Professional. pp. 901. ISBN 0-07-147247-9.
- Tietz N.W., (Ed.), Textbook of Clinical Chemistry, W.B. Saunders, (1986), p.1350.
- Moorehead, W.R. and Briggs, H.C., Clinical Chem., 20, 1458 (1974).

Pack Presentation

| | |
|-----------------------------|-------------|
| Product Code/ Catalogue No. | KGCO105.1.1 |
| Pack Size* | 2X50ml |
| Reagent 1 | 1X50ml |
| Reagent 2 | 1X50ml |
| Reagent 3 (Washing soln.) | 1X50ml |
| Reagent 4 | 1X2ml |











* Pack size may vary on market demand.

Revision No: (Ver: KGCO106.1/1)

Date of Issue: 1st April 2010

Symbols

Following symbols are used in the labeling of KEE GAD kits:

| | | | |
|---|----------------------|---|---------------------|
|  | Catalogue No. |  | Batch No. |
|  | CE Mark |  | Read instructions |
|  | In Vitro Diagnostics |  | Storage temperature |
|  | Expiry Date |  | Content |
|  | Product Name |  | Manufactured By |



Manufactured by:
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