INTRODUCTION

1. AutoZyme Calcium is a reagent set for determination of calcium based on the **colorimetric method using o-Cresolphthalein Complexone.**
2. AutoZyme Calcium is a **single reagent system,** using one step procedure.
3. AutoZyme Calcium is **linear** upto 15 mg%.
4. AutoZyme Calcium is a **High Stability Reagent.**
5. AutoZyme Calcium can be used on any **Colorimeter, Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
6. AutoZyme Calcium has **one step reconstitution.** It involves mixing of CPC and Diluent reagent.
7. Calcium can be determined in just **5 minutes.**
8. AutoZyme Calcium has **minimum reagent wastage** since working solution can be prepared according to the need.
9. **Interference of Mg and Fe is negligible.** Lipemic samples do not require any special treatment.

PRINCIPLE

Calcium forms a purple colour complex with cresolphthalein complexone in alkaline medium. This complex absorbs light at 575 (570 - 580) nm. The intensity of the colour is directly proportional to the calcium concentration in specimen.

Calcium Cresolphthalein Complexone $\xrightarrow{\text{Alkaline pH}}$ **Purple Complex**

PREPARATION OF WORKING SOLUTION

Prepare working solution by mixing equal volume of **CPC Reagent** and **Diluent Reagent.**

REAGENT STORAGE & STABILITY

The reagents are stable till the expiry date stated on the bottle label, when stored at 2-8°C.

The working solution is stable for 7 days at 2 - 8°C.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Diethanolamine buffer, pH 10.7	500 mmol/l
• O-Cresolphthalein Complexone	63 µmol/l
• Quinolinol	17 mmol/l
• Preservatives and Stabilizers	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Plastic or siliconized container should be avoided as it may prolong clotting time. Serum is preferred but heparinised plasma (200 IU/ml blood) can also be used. EDTA, Citrate, Oxalate and Calcium salt of heparin interfere in assay and should not be used as anticoagulants.

Calcium is stable in serum or plasma for 5 days when stored at 2 - 8°C and 20 days when stored at -10°C.

PROCEDURE

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** ..... **5 mins. at R.T. (25 - 30° C)**
- ☐ **Wavelength** ..... **575 nm.(570 - 58 0nm.)**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Sample volume** ..... **0.02 ml (20 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **10 mg%**
- ☐ **Linearity** ..... **15 mg/dl**

Manual assay procedure

Prewarm at room temperature (25 - 30°C) the required amount of working solution. Perform the assay as given below.

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.02 ml	0.02 ml	—
Working solution	1.0 ml	1.0 ml	1.0 ml

Incubation

Mix and keep the assay mixture for 5 minutes at room temperature (25 - 30°C). Measure the absorbance against blank at 575 nm. (570 - 580 nm.). Final colour is stable for one hour if not exposed to direct light.

Calculation:

$$\text{Calcium in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 10$$

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NOTE :

1. Avoid the contamination of reagent into standard during its repeated use.

2. **Glassware is the most common source of contamination in the calcium assay. It is strongly recommended that glassware required for assay be rinsed with 0.1 N HCl followed by repeated rinsing with demineralized water.**

3. The specimen and reagent volumes can proportionally be altered without affecting the final results.

EXPECTED VALUES

Expected value : 8.5 to 11.0 mg%

Expected range varies from population to population and each laboratory should establish its own normal range.

PROCEDURE LIMITATIONS

1. Patients receiving EDTA treatment can not be assayed for calcium correctly.
2. If the calcium value exceeds 15 mg% a suitable dilution can be made with normal saline. In such case the result obtained should be multiplied with the dilution factor to obtain the correct calcium value.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

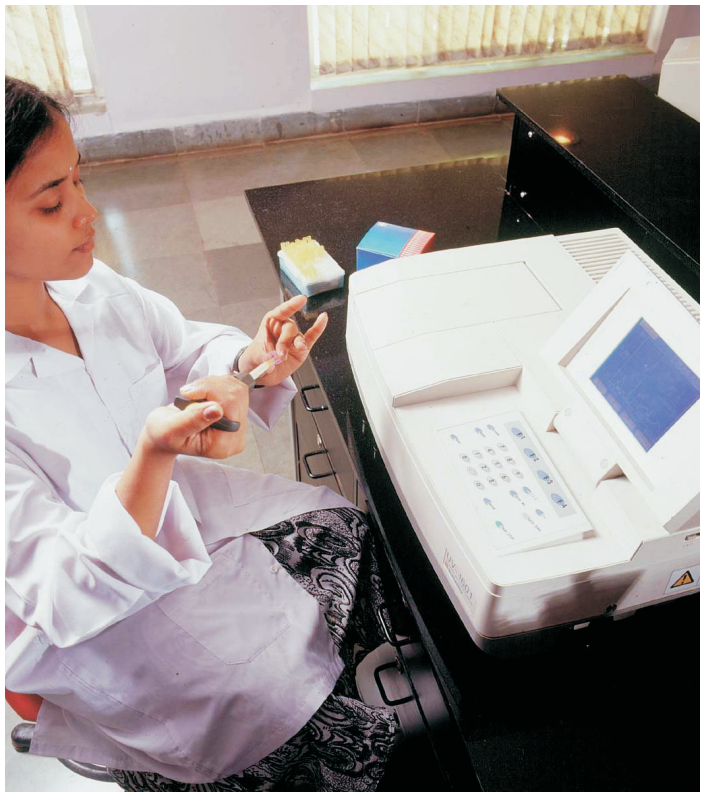
REFERENCES

1. Kessler G. et al, ***Clin. Chem.*** **10** 686 (1964).
2. Harold Varley,***"Practical Clinical Biochemistry"*** V. ed. pp. 858.

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE
European Conformity

AR. No.: I 06 *CA-2009-03-00I*



Quality Assurance - On line testing

AutoZyme

CALCIUM

Colorimetric

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