



CALCIUM AND MAGNESIUM DETERMINATION

OBJECTIVES

1. Apply the correct technique in the performance of calcium measurement in a given sample.
2. Analyze the laboratory results in terms of acceptability, accuracy, precision, and reliability.
3. Identify sources of error that may lead to false elevation or decrease in the calcium level.
4. Correlate laboratory results with clinical significance.

CALCIUM DETERMINATION

Materials

- Spectrophotometer and Cuvets
- Tissue
- Parafilm
- Serological pipets/automatic pipets
- Pipet tips
- Rubber aspirator
- Specimens (Control and unknown – serum or plasma)
- Test tube
- Centrifuge
- Calcium reagent kit

Reagents

BUFFER	LYSINE BUFFER (pH 11.1)
Reagent	Sodium azide
	8-Hydroxyquinoline
	O-Cresolphthalein complexone
	Hydrochloric acid
Standard	Calcium: 8 mg/dL or 2 mmol/L
	Sodium azide

Principle of the Method

- Calcium ions react with o-cresolphthalein complexone in an alkaline medium to form a purple colored complex. The absorbance of this complex is proportional to the calcium concentration in the sample.

Assay Requirements

- Wavelength – 570 nm
- Optical path – 1 cm
- Temperature – 37°C
- Measurement – against water blank
- Preparation of Working Reagent:
 - Add color reagent to buffer in equal volumes as required and allow to stand for 10 minutes at room temperature before use.

Procedure

Pipetting Scheme: semi-micro

Pipet into cuvetts	B	S	C _s	C _p	U
Standard solution		0.02 mL			
Control normal serum			0.02 mL		
Control pathologic serum				0.02 mL	
Patient's serum					0.02 mL
Working reagent	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL

- Mix and measure the absorbance of sample (ΔA_{sx}) and standard (ΔA_{STD}) against the reagent blank within 5 to 50 minutes.

CALCULATION

$$C = 8 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \quad \text{in mg/dL}$$

$$C = 2 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \quad \text{in mmol/L}$$

- **Reference range:** 2.15 – 2.50 mmol/L
8.8– 10 mg/dL

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