

**Reagent kit for the determination of calcium concentration in serum and urine.**

In the human body 98 - 99% of calcium is present in bound form in bones and teeth. About 50% of the blood calcium circulates in ionic form, the other part as bound to proteins. The concentration of ionic calcium is influenced by the acid-base household of the body. The ratio of ionic/protein-bound calcium is higher in acidosis and lower in alkalosis. Elevated calcium levels are found in association with primary hyperparathyroidism, neoplastic diseases (eg. breast cancer, bronchial cancer, pancreatic tumor), osteoporosis, Paget's disease and Addison's disease, overdosage of the vitamins A and D, hyperthyroidism. Lower calcium values are measured in hypoparathyroidism, disturbances of the absorption, chronic renal failure, nephrotic syndrome, hepatic cirrhosis, acute pancreatitis.

**Principle**

At a neutral pH, the Ca<sup>2+</sup> forms with arsenazo III a complex, the color intensity of which is directly proportional to the concentration of calcium in the sample.

**Reference values**

**Serum:** 2.2-2.55 mmol/l  
(8,80-10,2 mg/dl)  
**Urine:** 2.5- 8.0 mmol/24 h  
(100-320 mg/24 h)

It is recommended that each laboratory should assign its own normal range.

**Reagents compositions**

**1. Reagent (R1)**

MES, pH= 6.50 100 mmol/l  
Arsenazo III 200 µmol/l

**2. Calcium standard**

Ready for use. For details please check the insert.  
Available only in Cat. No.: 43941S

**Samples**

Serum free of haemolysis.  
Urine diluted in ratio of 1:3 with distilled water; adjust to pH 3-4 with 0.1N HCl.

**PROCEDURE**

**In the course of determination please use disposable plastic equipments only!**

**Preparation and stability of the working reagent**

The reagent is ready for use.  
If the absorbance of working reagent is higher than 1.5 at 600 nm the reagent can not be used.

**Assay Conditions**

Wavelength: 600 nm  
Temperature: 37 °C  
Cuvette: 1 cm light path  
Method: endpoint (increasing)  
Read against: reagent blank

**Pipette into cuvette**

	<i>Blank</i>	<i>Standard</i>	<i>Sample</i>
<i>Reagent</i>	1 ml	1 ml	1 ml
<i>Distilled water</i>	10 µl		
<i>Standard</i>		10 µl	
<i>Sample</i>			10 µl

Mix and read the optical density after a 1-minute incubation.

**Calibration (37°C, arsenazo method)**

S1: Distilled water  
S2: Calcium standard Cat. No.: 52001 or  
Roche C.F.A.S. (Calibrator for automated system)  
Randox Calibration Serum Level I

**Calibration frequency**

Two point calibration is recommended  
- after reagent lot change,

- as required following quality control procedures.

**Calculation using calibration**

$$\frac{A_{sample}}{A_{standard}} \times C_{standard} = C_{sample}$$

A=absorbance  
C=concentration

**Quality control**

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**PERFORMANCES DATA**

The following data were obtained using the Olympus 600 analyzer.

**Linearity**

Up to 4 mmol/l (16,0 mg/dl).

**Sensitivity**

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.013 mmol/l (0,052 mg/dl) Calcium concentration at 600 nm.

**Precision**

	<b>Reproducibility</b>		
	Average conc. (mmol/l)	SD	CV%
<b>Sample I.</b>	2.21	0.044	1.98
<b>Sample II.</b>	3.48	0.071	2.04

**Correlation**

Comparative studies were done to compare our reagent with another commercial calcium assay.

The results from these studies are detailed below.

Correlation coefficient: r=0.988

Linear regression: y(mmol/l)= 1.052x+0.003

(x= other commercial reagent, y= own reagent).

**Specificity**

Bilirubin 855 µmol/l (50mg/dl), lipid 700mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.

**Note**

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

**For in vitro diagnostic use only.**

**The following symbols are used on labels**

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

**Bibliography**

Baver, P. J.,: Anal. Biochem, 110; 61, (1981)