

# COMPARISON OF SERUM CALCIUM ESTIMATION BY DIRECT COLORIMETRIC AND VOLUME / VOLUME COLORIMETRIC METHODS BASED ON O-CRESOLPHTALEIN PRINCIPLE

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## ABSTRACT

*Introduction:* Calcium is the most abundant mineral and fifth most common element in the body. There are many different methods for estimation of serum calcium like spectrophotometric, ion selective electrode (ISE) and atomic absorption methods. *Objective:* Aim of our study was to compare serum calcium estimation by using direct colorimetric and volume / volume colorimetric (v/v) methods based on o-Cresolphthalein principle. *Materials and Methods:* The study was performed in a tertiary care laboratory in Rawalpindi from March to June '2011. It was a comparative prospective study. Seventy commercial quality control samples of Randox laboratories were simultaneously tested by both the methods. Results were analyzed on SPSS version 17. *Results:* In normal controls, the v/v method kit gave the mean result of 2.34 mmol/L  $\pm$  0.04 with a CV of 1.70% while with the direct colorimetric method kit the values were 2.31 mmol/L  $\pm$  0.03 with a CV of 1.30%. In abnormal controls, v/v method kit gave the mean value to be 3.09 mmol/L  $\pm$  0.04 with a CV of 1.29% while with the direct colorimetric method kit, the mean value was 3.07 mmol/L  $\pm$  0.03 with a CV of 0.97%. *Conclusion:* Both the kits are recommended for use in a tertiary care laboratory as the precision and accuracy of both kits is comparable. But the volume / volume colorimetric kit is more cost effective.

## INTRODUCTION

Calcium is the most abundant mineral<sup>1</sup> and fifth most common element in the body.<sup>2</sup> Almost all blood calcium is present in plasma and reference range is 2.10 to 2.65 mmol/L. It is present as free or ionized (50%), protein bound usually with albumin (40%) and complexes with small anions (10%). Calcium is needed for bone mineralisation, blood coagulation and influences the permeability and excitation of plasma membranes. It is usually monitored for hypoparathyroidism, hyperparathyroidism, vitamin D deficiency, malnutrition, cancers, enhanced renal retention, osteoporosis, etc.

There are many different methods for estimation of serum calcium like spectrophotometric, ion selective electrode (ISE) and atomic absorption methods. The spectrophotometric techniques use metallochromic indicators which change color when they bind to calcium. Arsenazo III and o-Cresolphthalein Complexone (CPC) methods are the two spectrophotometric techniques frequently used.

Aim of our study was to compare serum calcium estimation by CPC method using direct colorimetric and volume / volume colorimetric (v/v) methods. The principle of CPC method is that calcium reacts with CPC in an alkaline medium to form a red coloured complex. This complex is measured at a wavelength at 570 nm. Sample is diluted with acid to re-

lease protein bound and complexed calcium. Diethylamine, 2-amino-2-methyl-1-propranolol or 2-ethylaminoethanol is added to buffer the solution and provide an alkaline medium.<sup>3</sup> Effect of magnesium can be minimised either by adding 8-hydroxyquinolone, buffering the solution to pH of around 12 or by measuring absorbance at 580 nm.<sup>4</sup>

## MATERIALS AND METHODS

The study was performed in a tertiary care laboratory in Rawalpindi from March to June '2011. It was a prospective comparative study. Seventy quality control samples of Randox laboratories were used of these thirty five were normal controls while thirty five were abnormal controls. Controls were tested simultaneously on both the kits provided by SS diagnostics using a fully automated chemistry analyser (Selectra E). Data was recorded using specially designed proformas and results were analysed using SPSS version 17.

## RESULTS

Our results showed that in normal control samples with a target of 2.33 mmol/L where the range of calcium was 2.10 to 2.56 mmol/L. The v/v method kit gave the mean result of 2.34 mmol/L  $\pm$  0.04 with a CV of 1.70%. With the direct calorimetric

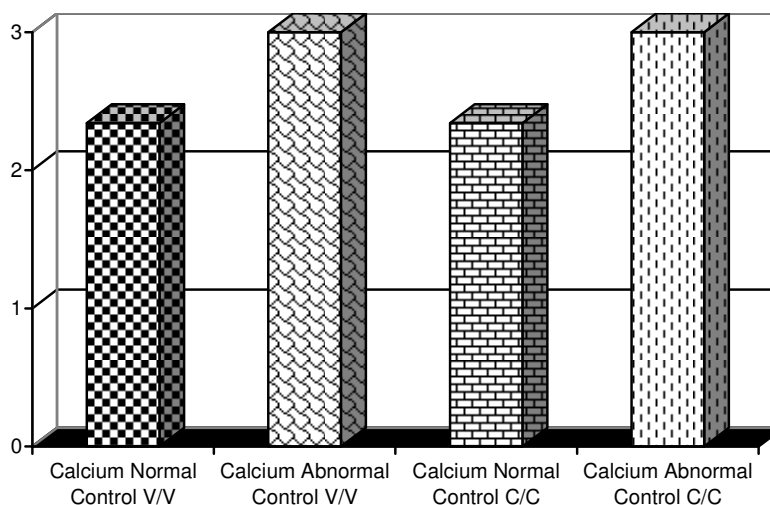
method, the values were 2.31 mmol/L  $\pm$  0.03 with a CV of 1.30% (Table 1 and Figure 1). The abnormal controls had a range of 2.76 to 3.38 mmol/L with a target range of 3.07 mmol/L. The v/v method gave the mean value to be 3.09 mmol/L  $\pm$  0.04 with a CV of 1.29%. With the direct calorimetric method kit, the mean value was 3.07 mmol/L  $\pm$  0.03 with a CV of 0.97% (Table 1 and Fig. 1). Results show that both the kits give comparable results at both normal and high values. Fig. 2 gives the box plot for normal and abnormal quality controls for both the kits.

**DISCUSSION**

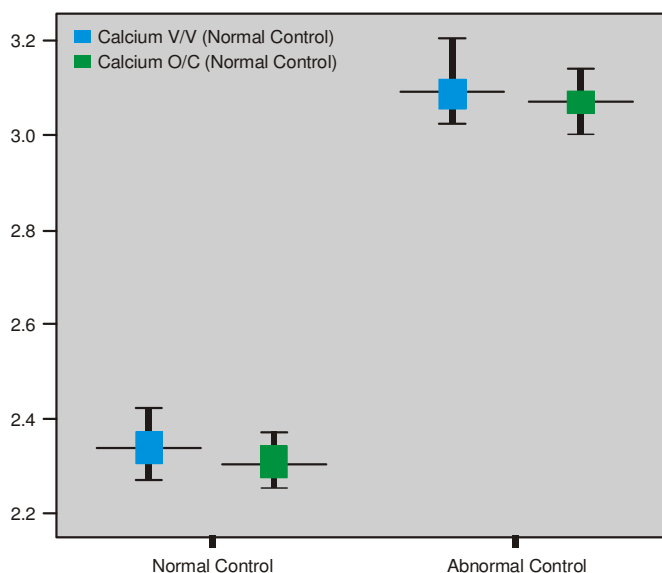
There are many different analytical techniques for the estimation of calcium. When we are comparing 2 different methods for the same principle we have to see the precision and accuracy, ease with which the test can be done and the cost of the reagent. Both the kits use the same principle that is CPC reaction with calcium gives a red colour in an alkaline medium. Both the kits have 2 reagents, R<sub>1</sub> which is the buffer and contains ethanalamine and R<sub>2</sub> which is the chromogen and contains 0.62 mmol/L of o-Cresolphtalein and 69 mmol/L of 8-Hidroxyquinolone. In the v/v method reagents are ready to use while in the direct calorimetric method we have to make a monoreagent which is made by mixing fifty volumes of R<sub>1</sub> and one volume of R<sub>2</sub>. In both the kits the reagents remain stable till the expiry date when stored in a tightly closed container at 2 – 8°C and protected from light. Both the kits can be used for carrying out test on serum or plasma as well as on urine specimens. In the v/v method kit a calibrator is used while in direct calorimetric method kit standard is used. In v/v method, 1 mL of both R<sub>1</sub> and R<sub>2</sub> are put in the blank, calibrator and sample cuvettes. Then we add 20 uL of calibrator in the calibrator cuvette while 20 uL of sample in the sample cuvette. While in the direct calorimetric method we put 2 mL of R<sub>1</sub> in blank, standard and sample cuvette. Then we add 1 drop of R<sub>2</sub> with help of a dropper in all 3 cuvettes. While adding 1 drop of R<sub>2</sub>, special care is taken and here the operator skill is also important. Then we add 20 uL of standard in standard cuvette while 20 uL of sample in sample cuvette. Then these cuvettes are mixed and incubated for 5 minutes at 37°C, after which absor-

**Table 1:** Calcium normal and abnormal control results between V/V and direct colorimetric O/C methods.

Methods	Normal Control n = 35 (2.10 – 2.56 mmol/L) Target 2.33 mmol/L		Abnormal Control n = 35 (2.76 – 3.38 mmol/L) Target 3.07 mmol/L	
	Mean $\pm$ SD	CV%	Mean $\pm$ SD	CV%
Calcium V/V	2.34 $\pm$ 0.04	1.70	3.09 $\pm$ 0.04	1.29
Calcium O/C	2.31 $\pm$ 0.03	1.30	3.07 $\pm$ 0.03	0.97



**Fig. 1:** Bar chart showing normal and abnormal control value for V/V and O/C methods.



**Fig. 2:** Box plot showing normal and abnormal control for V/V and direct colorimetric O/C methods.

balance is taken. Calcium v/v kit is more cost effective as compared to direct colorimetric Calcium o/c kit.

In *conclusion* depending upon the results, both the kits are recommended for use in a tertiary care laboratory as accuracy and precision of both kits is comparable. Although the direct calorimetric kit is more cost effective but it requires more operator skill.

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