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An evaluation of precision and accuracy of calciumreagent kits by the procedure of Vikelsöe et al

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The procedure suggested by Vikelsöe et al., for evaluating the performance characteristics of analytical methods was employed to estimate simultaneously the precision and accuracy of 3 calcium reagent kits. Calcium binding to ortho-cresolphthalein complexone is the principle chemical reaction utilized by all kits evaluated. Although slight differences in the degree of imprecision of 3 reagent kits were demonstrated, these values are acceptable for clinical interpretation of serum calcium. The precision dose profiles expressed as percent coefficient of variation (%, CV), at calcium concentrations 9.6, 10.4, 11.2 and 12.0 mg/dl, of the 3 reagent kits were 1.13, 1.44, 1.31 and 1.32% for kit I; 2.03, 1.78, 1.53 and 1.13% for kit II; 1.16, 1.90, 1.29 and 1.22% for kit III, respectively. Moreover, the relative accuracy of each kit was identified by comparing with the designated values (9.6 - 12.0 mg/dl) for calcium (X) in the quality control serum. The linear regression analyses of the 3 calcium reagent kits (Y) were as follow : $Y = -1.712 + 1.203\ X$ ($r = 0.980$), $Y = 2.48 + 0.669\ X$ ($r = 0.976$) and $Y = 1.131 + 0.861\ X$ ($r = 0.996$). The slight differences in systematic error of calcium values, estimated by 3 calcium reagent kits, were observed at the concentrations studied. Types and analytical ranges of the control material may affect the accuracy study.

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สมพงษ์ จินายน, ประสาท อักษรวงศ์. ประเมินผลความเที่ยงตรงและแม่นยำของน้ำยาสำเร็จรูปที่ใช้วัดปริมาณแคลเซียม โดยวิธีของ Vikelsöe และคณะ. จุฬาลงกรณ์เวชสาร 2531 กรกฎาคม; 32(7) : 625-630

ได้ประเมินคุณสมบัติด้านการปฏิบัติของวิธีวัดปริมาณแคลเซียมโดยน้ำยาสำเร็จรูปด้วยการนำวิธีของ Vikelsöe และคณะ มาใช้ทดสอบความเที่ยงตรงและแม่นยำ ที่หลายระดับความเข้มข้นได้พร้อมกันในการทดลองเดียวกัน น้ำยาทั้ง 3 ชนิดใช้หลักการรวมตัวระหว่างแคลเซียมกับสี ortho-cresolphthalein complexone พบว่าน้ำยาแต่ละชนิดมีความเที่ยงตรงแตกต่างกันเล็กน้อย และอยู่ในเกณฑ์ที่ยอมรับได้สำหรับการแปลผลการตรวจแคลเซียมทางคลินิก ค่าความเที่ยงตรง (%, CV) ที่ระดับความเข้มข้นแคลเซียม 9.6, 10.4, 11.2 และ 12.0 มก/ดล สำหรับน้ำยาสำเร็จรูปทั้ง 3 ชนิด คือชนิดที่หนึ่ง 1.13, 1.44, 1.31 และ 1.32% ชนิดที่สอง 2.03, 1.78, 1.53 และ 1.13% ชนิดที่สาม 1.16, 1.90, 1.29 และ 1.22% ตามลำดับ นอกจากนี้ยังพบความแตกต่างกันเล็กน้อยในแง่ความแม่นยำสัมพันธ์ด้วย ทั้งนี้โดยเปรียบเทียบกับค่าแคลเซียมที่กำหนดไว้ (X) ในวัตถุควบคุม (9.6 - 12.0 มก/ดล) สมการความถดถอยเชิงเส้นตรงของน้ำยาสำเร็จรูปทั้ง 3 ชนิด แสดงตามลำดับ คือ $Y = -1.712 + 1.203 X$ ($r = 0.980$), $Y = 2.48 + 0.669 X$ ($r = 0.976$) และ $Y = 1.131 + 0.861 X$ ($r = 0.996$) ในแง่ความแม่นยำของค่าวิเคราะห์นั้น ชนิดของวัตถุควบคุมและระดับความเข้มข้นของแคลเซียมอาจมีผลกระทบต่อผลการศึกษาได้

Precision and accuracy are analytical performances to be considered in order to implement an analytical technic as a routine laboratory test.[1] Precision dose profiles and accuracy studies are usually designed as two separate experiments.[2] Moreover, method comparison is the most informative procedure to detect any inaccuracy of a proposed method.[3] Occasionally a small size laboratory may experience some difficuties in establishing or in seeking an appropriate reference method to be used as a comparative method. Vikelsöe et al.[4] had introduced an evaluation program for simultaneously determining precision and accuracy of an analytical method by which a reference or a comparative method is not required. Moreover, a laboratory with limited budget should be able to perform the evaluation studies. The program suggested by Vikelsöe et al. was employed as a single experiment to estimate the precision and accuracy of 2 creatinine methods.[5] Jaffe reaction is the principle chemical reaction for both manual methods : endpoint colorimetric and kinetic colorimetric assays. Commercial control sera with stated analytical values were repeatedly analysed by the test methods. The results were then calculated for coefficient of variation (CV, %) and least squares analysis which indicated that the accuracy of the two methods was comparable, eventhough the kinetic colorimetry tended to be more imprecise than the endpoint colorimetric one.[5]

The aim of this study is to use the Vikelsöe et al. procedure to estimate the precision dose profiles as well as the accuracy of three commercial calcium kits. All employ manual spectrometry and dye-binding properties of calcium with orthocresolphthalein complexone.

Materials and method

Commercial calcium kits were assigned as kit I, II and III. Biological materials used as references to study the performance characteristics of analytical methods were products of Gilford Irvine Company (California, 92714, U.S.A.).They were the QCS normal control serum assayed (N), product code 9702, lot number 020 B 02 and abnormal control serum assayed (H), product code 9705, lot number 025 B 02. The designated values of calcium determined by atomic absorption spectrophotometry in the two control sera were 9.6 and 12.0 mg/dl, respectively. For the evaluation procedure, a group of 10 determinations was performed for each of the 4 samples prepared by mixing known ratios by volume of two pools of the control sera (N, 2/3 N + 1/3 H, 1/3 N + 2/3 H, H, ml). The actual final concentrations of the calcium in these specimens were 9.6, 10.4, 11.2 and 12.0 mg/dl.

The calcium values of the same prepared specimens were analysed by 3 calcium kits with calcium standards provided and by following the method directions supplied. For spectrophotometry, a Coleman Junior II spectrophotometer was used, set at 570 nm wavelength. The glasswares were cleaned with 1 mol/L hydrochloric acid solution, followed by distilled water, to remove any contaminating calcium.

Statistical calculations were performed on a scientific hand-held calculator to obtain the group means, standard deviations and coefficient of variations (CV, %) for the estimation of imprecision. The least squares regression analysis was performed on the group means and designated calcium values to yield the slope and the intercept of the straight line for the estimation of accuracy. The standard deviation of the residual (Sy.x) and correlation coefficient (r) were also computed, as well as the deviations from linearity.[4,5,6]

Result

Three calcium kits evaluated show slight differences in precision dose profiles of 4 calcium levels ranging from 9.6 to 12.0 mg/dl as shown in table 1. In figure 1, the CVs, % are plotted as the function of calcium doses. The precision dose profile curves of kit I and Kit III showed similar patterns for all concentrations studied. For kit II the precision at normal calcium levels was lower than those at high calcium levels.

The accuracy of calcium values was estimated by means of the following two parameters : The slope of the regression line, and the deviation from linearity (Y observation − Y regression). These parameters will tend towards 1 and 0 respectively, with increasing accuracy. The results are demonstrated in table 1 and figure 2. By observing the values for slope, the relative accuracy of analytical results of kit I, kit II and kit III was compared. Considering the difference between group mean values (Y observation) and corresponding regression line values (Y regression) - in mg/dl as the function of calcium concentration (mg/dl), all 3 calcium kits had comparable results (figure 2).

Discussion

The program of Vikelsöe et al. (1974) proved to be a suitable method for the initial evaluation of the analytical performances of 2 creatinine methods,[5,6] and of 3 calcium kits in this study. As performance characteristics of laboratory technics are essential for analysis of patients' sera, these should be implemented. The reagent kits are inexpensive and available for

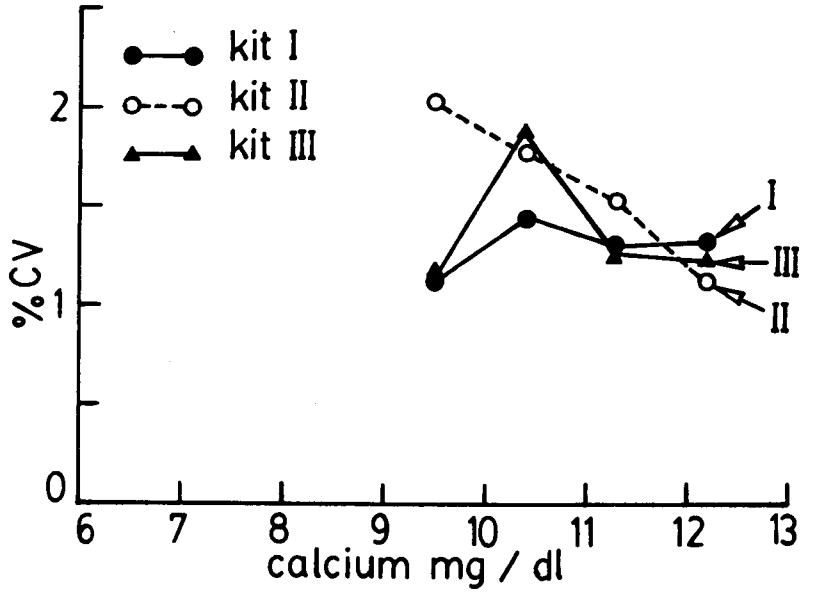

Figure 1 Precision dose profiles of 3 calcium kits.

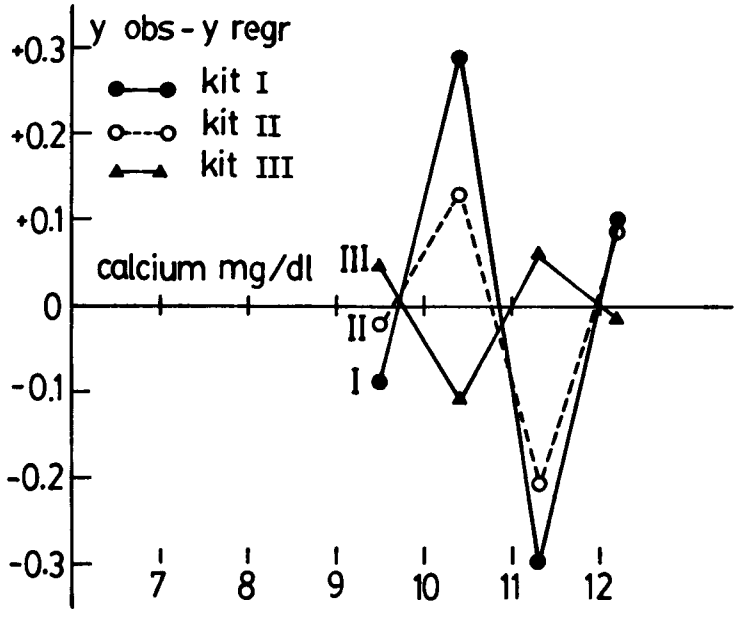

Figure 2 Deviation from linearity (Y observation – Y regression) of 3 calcium kits.

Table 1 Data comparing precision and accuracy of the 3 calcium kits.

test material	kit I		kit II		kit III	
	mean ± SD	CV,%	mean ± SD	CV,%	mean ± SD	CV,%
designated value : (mg/dl)						
9.6	9.74±0.11	1.13	8.88±0.18	2.03	9.45±0.11	1.16
10.4	11.08±0.16	1.44	9.57±0.17	1.78	9.98±0.19	1.90
11.2	11.46±0.15	1.31	9.76±0.15	1.53	10.84±0.14	1.29
12.0	12.82±0.17	1.32	10.60±0.12	1.13	11.46±0.14	1.22
Regression analysis :						
equation	y = −1.712 + 1.203x		y = 2.48 + 0.669x		y = 1.131 + 0.861x	
slope	1.203		0.669		0.861	
Sy. x	0.253		0.152		0.078	
Correlation coefficient (r) :	0.980		0.976		0.996	

routine laboratory use either at peripheral health care units or district hospitals.

The principle chemical reaction of all 3 calcium kits is a metal complexing dye, orthocresolphthalein complexone (OCPC) in an alkali buffer (pH 11.0±0.1) to which calcium is bound to produce a purple coloured chromophore having absorbance at wavalength 570 nm.[7] This principle is widely used elsewhere to quantitate calcium in the blood by both manual analyses[7,8] and by automations.[9,10,11] It also shows good performance characteristics with respect to precision and accuracy. Magnesium interference is overcome by the addition of 8-hydroxyquinoline.

By using the evaluation program of Vikelsöe et al., the data of precision dose profiles of the 3 calcium kits were within the routine condition variance (CV, 2.5 %) of laboratory for calcium analysis[12] and within a medical significant value of calcium (2.27%) suggested by Barnett.[13] The differences between % CV of each kit at the same calcium concentration prepared were minimal. However, the range tested was 9.6-12.0 g/dl, the lower and higher concentrations need to be further evaluated. We did not use pure calcium solutions in this experiment, because commercial sera have viscosity equivalent to patients' specimens. Our previous experiment had demonstrated the effect of sample matrix on the precision dose profiles of creatinine.[5] The relative accuracy was considered by 2 parameters, from regression analysis : slope, and deviation from linearity The calcium values assigned to the test materials were obtained by atomic absorption spectrophotometry which has been recommended as a reference method for the analysis of serum or plasma calcium.[14] The results of statistical analysis indicates that calcium values estimated by 3 kits yielded slight systematic analytical errors, for the concentrations assayed (9.6 - 12.0 mg/dl). From the data available, a conclusion regarding accuracy can not as yet be determined, and awaits other types of reference material with more expanded ranges of designated calcium values to be used in method evaluation experiments. However, the program suggested by Vikelsöe et al.[4] can be effectively used in the initial step of method evaluation for a one day or a long-term experiment.[5,6] Also manufacturers should provide users with data of performance characteristics as well as the application and methodology characteristics.

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