



# Intra- and interindividual biological variability of total calcium, urea, and creatinine in pre-dialysis and post-dialysis adult patients with chronic renal failure

Cristina Paola Vernaza Guerra\*<sup>1</sup>, Lourdes Alicia Pazmiño Martínez<sup>1</sup>

1. Carrera de Bioquímica Clínica, Facultad de Ciencias Médicas, Universidad Central del Ecuador, Quito-Ecuador.

## Abstract

**Introduction:** The intra- and interindividual variability of calcium, urea, and creatinine in hemodialysis patients depend on the nutritional status and the removal produced by the hemodialysis treatment. The objective of the present study was to determine the variability in a 10-month observation period in a group of patients in a hemodialysis program.

**Methods:** This longitudinal study was conducted with adult patients with stage 5-d chronic kidney disease from the Clinef-Norte dialysis center in Quito-Ecuador. The variables were age, sex, urea, creatinine, and total calcium before and after dialysis. The sample was probabilistic, of 120 cases. The individual biological variation (CVI), the inter-individual biological variation (CVG), the change reference value, and the relative variation coefficient (CVR) were calculated.

**Results:** 165 cases,  $60.5 \pm 15.2$  years old, were analyzed. The CVI of urea was 0.36% and CVG of 0.87% in pre-dialysis, CVI of 24.3%, and CVG of 28.94% in post-dialysis. Creatinine CVI was 10.8% and CVG 24.23% pre-dialysis, post-dialysis CVI 12.9%, and CVG 36.36%. The CVI of Calcium was not calculable; the CVG was 7.31%.


**Conclusions:** The CVI of pre-dialysis urea indicated a regular protein intake. The CVI of post-dialysis urea was variable and is related to differences in the prescription of the extracorporeal flow of hemodialysis. The CVI of serum creatinine has implications for constant loss of muscle mass in the study time. Calcium GVC was associated with an increase in calcaemia in the first four months and a continuous fall after this period, probably associated with a decrease in the intake of calcium products after the fourth month of dialysis treatment.

## Keywords:

**MESH:** Kidney Failure, Chronic; Renal Insufficiency, Chronic; Renal Dialysis; Calcium; Blood Urea Nitrogen; Creatinine; Biological Variation, Population.

**Received:** November 12, 2021  
**Accepted:** January 9, 2022  
**Published:** February 28, 2022  
**Editor:** Dr. Franklin Mora Bravo.

**Cite:**  
Vernaza C, Pazmiño L. Intra- and interindividual biological variability of total calcium, urea, and creatinine in pre-dialysis and post-dialysis adult patients with chronic renal failure. *REVSEN* 2022;10(1):13-20.

 Copyright Vernaza C, et al. This article is distributed under [Creative Commons CC BY-NC-SA 4.0 Attribution License](https://creativecommons.org/licenses/by/4.0/), which allows the use and redistribution citing the source and the original author for non-commercial purposes.

\* Corresponding author



Intra- and interindividual variability of calcium, urea, and creatinine in hemodialysis patients depends on the nutritional status and the removal produced by the hemodialysis treatment. Usually, a patient who starts a hemodialysis program presents with hypocalcemia and uremia with variable creatinine levels depending on muscle mass. With constant and dosed removal maintained by a three-week hemodialysis program, equilibrium is usually reached in the first semester once the program has started, with definitive vascular access functioning and nutritional recovery. Once a state of nutritional equilibrium occurs, the loss and gain of urea, creatinine, and calcium have a predictable variability in the interdialytic period due to the balance between metabolic turnover and homeostatic-dialytic regulation and, together with the critical delta, allows determining if the change between the two values in a patient is clinically significant in the face of new events such as infection or acute malnutrition. The intraindividual coefficient of variation, imprecision, or semiannual random error of the method used in the laboratory or the measurement procedure must be calculated [1]. To ensure the quality of the results, the laboratory must carefully monitor the performance of the different methods by implementing a quality control program, which includes the processing and analysis of internal controls and participation in external quality assessment programs for each of the determinations that it performs must be evaluated through the Analytical Variation (AV) according to the procedures and the methodology used, taking into account the precision and accuracy of the methods [2]. When we carry out serial laboratory tests to monitor a patient's health status, the doctor must know all the elements that can be sources of variation in the measurements, excluding the lack of harmonization of the techniques used for the determinations [3]. However, it should be noted that the intraindividual biological variability is much smaller than the interindividual biological variability [1].

The term Reference Variation of Change (RCV) "was introduced by Harris and Yasaka (1983) to identify significant changes in the status of patients during monitoring of their diseases" [4]. In April 1999, an international meeting was held in Stockholm to achieve a consensus regarding the hierarchy of each of the valid proposals to establish analytical quality criteria, as a result of which a database was published that is updated every two years, with sufficient information on the intra- and interindividual biological variability of a large part of the substances that are determined in the clinical laboratory [5, 6]; however, there are very few data on established intraindividual biological variability in the Ecuadorian population for patients with some nosological entity, and among them the patients with chronic renal insufficiency (CRF), there are few studies with low

statistical performance that allow establishing the range of values accepted or established for this disease considering the variability that the measurements of the method used present, for which reason the present study focuses on these measurements in the population in hemodialysis with variables that influence the obtaining of results on the calculations of the coefficient of biological variation, analytical variation, and the VRC.

## Materials and methods

### Study design

The present study is longitudinal.

### Scenery

The study was carried out at the NETLAB clinical laboratory service in Quito-Ecuador. The study period was from March 1, 2017, to March 31, 2018, and the study ended on July 30, 2018.

### Participants

Adult patients older than or equal to 18 years with stage 5-D CRF who underwent pre- and postdialysis studies during the study period were included. The patients belonged to the Clinef Norte dialysis center. Patients who did not have a complete assessment in the period were excluded.

### Variables

The variables studied were age, sex, urea, creatinine, and total calcium before and after dialysis. Measurements were observed for ten consecutive months.

### Data sources/measurements

The samples were received in the preanalytical area of the Specialized Reference Laboratory NETLAB SA, Quito headquarters. The plasma samples obtained from the patients who underwent predialysis and postdialysis were collected in the respective tubes with heparin. They were processed in the Chemistry area in the Cobas 8000 c702 equipment with serial number 15E2-10 code 2441 using spectrophotometry.

The method for quantifying calcium was used with reagent 1 (R1) 3-(cyclohexylamine)-2-hydroxy-1-propane sulfonic acid, and under alkaline conditions, the human sample reacted with 5-nitro-5'-methyl-BAPTA, which led to a color change since they are nonreactive surfactants and stabilizers. An incubation phase was carried out in the equipment, and the calcium-NM-BAPTA complex was formed. A second step was carried out with reagent two (R2), made up of EDTA, which extracts the calcium



ion from the complex formed; the result of this reaction process that is released is measured photometrically at 340 nm with the colorimetric method.

In urea determination, 9% NaCl reagent and the reagent and a second reagent containing a TRIS plug and various elements plus preservatives were used; nonreactive stabilizers, care is taken to store at 2-8 °C to maintain stability. Then, when these reagents act, urea hydrolyzes with urease to form ammonium and carbonate to give rise to a second reaction with 2-oxoglutarate in the presence of glutamate dehydrogenase (GLDH) and its respective coenzyme (NADH) to produce L-glutamate, and as the reduction of the NADH concentration is directly proportional to the concentration of urea that the sample contains, this was determined by measuring its absorbance at 340 nm photometrically with the method that establishes the kinetics.

Creatinine was quantified using the colorimetric Jaffe kinetic method using potassium hydroxide as a preservative and stabilizer; then, with reagents two and three, which contain picric acid, a yellow–orange complex is formed. A blank should be used to minimize interference with samples that possibly contain bilirubin, so the formation of the dye should be proportional to the concentration of creatinine in the sample being analyzed by performing absorbance measurement spectrophotometrically.

### Biases

The results were validated by the technical operators and by a higher validation confirmation area (inspector). Reagents from the commercial house (Roche) were used for each of the analytes to be processed: for total calcium, urea, and creatinine; "Peci control clinched" multi 1 (normal) and "Peci control clinched multi 2 (pathological)" was used. And 45AmpollaS ISE Standard, PCTM1 and PCTM2: Low XN, and high XN, Anti HBS, normal and pathological, Diluent NaCl 9%, 50 mL. These control values were recorded on the Infinity QC system, compliant with Westgard rules.

### Studio size

The sample was probabilistic, with a confidence interval of 95% and a size of the study population of 200 patients, with an error of 5%; the sample calculation was 120 cases.

### Quantitative variables

With the values of urea, creatinine, and calcium, the following indices were calculated: Individuality index =  $[CVA2 + CV_i^2]^{1/2}/CV_G$ . A value greater than 0.6 was determined to be high, where  $CV_i$  is the intra individual biological variation and  $CV_G$  is the interindividual biological variation. Individual variance ( $S^2_{W+A}$ ), analytical variance ( $S^2_{A}$  mg/dl) and biological variation, urea (mg/dl), creatinine (mg/dl) and total calcium (mg/dl) pre-

and postdialysis per event. The interindividual variation ( $S^2_g$ ) is calculated from the total variance of all the samples - $ST$ - to which intraindividual variation ( $S^2_i$ ) is subtracted and is expressed as CV. Calculated from the Exchange Reference Value or "Delta Check" with the equation:

$$\sqrt{2 \times 1.96 \times (CV_i^2 + CV_{NL}^2)}$$

The relative coefficient of variation (RCV) =  $CV_{NL} / CV_i$  was calculated.

### Statistical analysis

A descriptive analysis is presented. Additionally, the variables on the scale are presented as the means and standard deviations. Means and deltas across monthly comparisons were analyzed with analysis of variance (ANOVA). The statistical program SPSS 18.0 for PC (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.)

## Results

### Participants

A total of 165 patients entered the study. The diagram of the participants is presented in Figure 1.

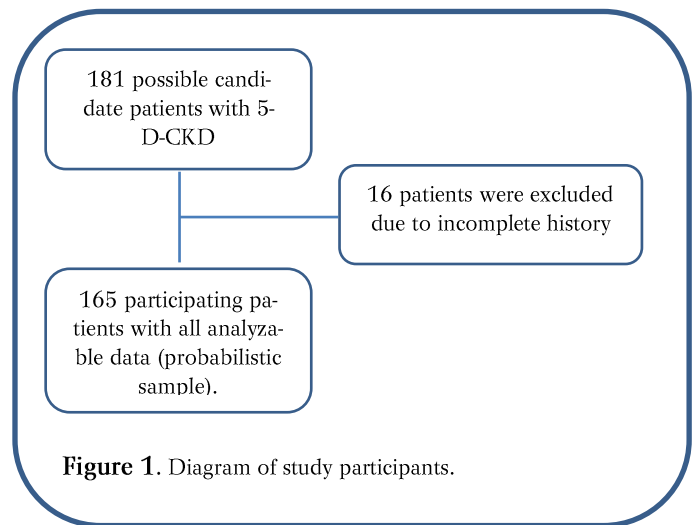


Figure 1. Diagram of study participants.

### Characteristics of the study population

The average age was  $60.5 \pm 15.2$  years (range: 21 – 94 years), with 52.7% (n=87) being male. The mean age of male subjects was  $62.6 \pm 14.9$  years, and for females, it was  $58.2 \pm 15.2$  years ( $P > 0.05$ ). The studies were performed with a separation time of  $29.6 \pm 1.9$  days; 10 continuous measurements (10 months) were performed in the group of patients. The average concentrations identified before and after dialysis are shown in Table 1, disaggregated by urea, creatinine, and calcium before and after dialysis. Serum creatinine is presented in Figure 2.

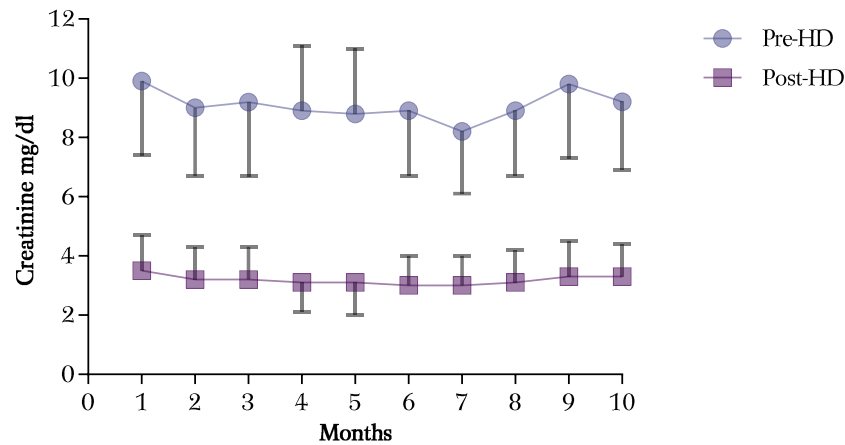


Figure 2. Serum creatinine value over time in the study group.

**Intraindividual variation**

The intraindividual biological variability, analytical, and biological variation are presented in Table 2. The intraindividual coefficient of variation for pre-HD urea was 0.36%, for creatinine, it was 10.8%, and for calcium, it was not calculable.

**Interindividual variation**

The interindividual variability is presented in table 3. The inter-individual coefficient of variation for pre-HD urea was 0.87%, for creatinine, it was 24.23%, and for calcium, it was 7.31%.

**Relative coefficient of variation**

The relative coefficient of variation (RCV) is presented in table 4. The RCV for pre-HD urea was 8.86%, for creatinine, it was 0.37%, and for calcium, it was not calculable. Comparisons of the coefficients with previously published studies are presented (Tables 5 and 6).

Table 1. Serum concentrations of urea, creatinine, and calcium before and after dialysis in the months of study.

	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month 10
Pre-HD urea (mg/ dL)	325.5 ±	325.5 ± 31	325.2 ± 30	325.2 ± 30	326.3 ± 36	324.0 ± 26	325.4 ± 30	325.5 ± 31	324.3 ± 29	325.5 ± 32
Post-HD urea (mg/ dL)	36.9 ± 13	31.2 ± 11	30.9 ± 13	30.7 ± 13	31.2 ± 10	28.5 ± 8.8	31.9 ± 9.6	30.6 ± 9.6	36.5 ± 13.2	29.2 ± 9.3
Creat pre-HD (mg/ dL)	9.9 ± 2.5	9.0 ± 2.3	9.2 ± 2.5	8.9 ± 2.2	8.8 ± 2.2	8.9 ± 2.2	8.2 ± 2.1	8.9 ± 2.2	9.8 ± 2.5	9.2 ± 2.3
Creat post-HD (mg/ dL)	3.5±1.2	3.2 ± 1.1	3.2 ± 1.1	3.1 ± 1.0	3.1 ± 1.1	3.0±1.0	3.0±1.0	3.1 ± 1.1	3.3 ± 1.2	3.3 ± 1.1
Calcium pre-HD (mg/ dL)	9.3 ± 0.8	8.8 ± 0.6	9.9 ± 0.6	9.9 ± 0.6	8.9 ± 0.6	9.0±0.6	8.9 ± 0.6	8.8 ± 0.6	8.9 ± 0.7	8.8 ± 0.6

Creat: Serum creatinine. HD: Hemodialysis.

Table 2. Intra -individual urea, creatinine, and calcium variation in the study group.

	individual variance	Analytical variance	biological variation	Intra - individual coefficient of variation
Pre-HD urea (mg/ dL)	138.90	2.84	136.06	0.36%
Post-HD urea (mg/ dL)	54.82	2.84	51.97	24.3%
Creat pre-HD (mg/ dL)	0.9838	0.092	0.89	10.8%
Creat post-HD (mg/ dL)	0.2275	0.092	0.14	12.9%
Calcium pre-HD (mg/ dL)	0.2135	0.24	-0.03	NC

NC. not calculable

**Table 3.** Interindividual variation of urea, creatinine, calcium in the study group.

	Total sample variance	Intra-individual variation	Interindividual variation	Interindividual coefficient of variation
Pre-HD urea (mg/dL)	944.34	136.06	808.28	0.87%
Post-HD urea (mg/dL)	125.34	51.97	73.37	28.94%
Creat pre-HD (mg/dL)	5.34	0.89	4.45	24.23%
Creat post-HD (mg/dL)	1.26	0.14	1.12	36.36%
Calcium pre-HD (mg/dL)	0.42	-0.03	0.42	7.31%

\* Assume  $S^2_i=0$ .**Table 4.** Relative coefficient of variation (RCV).

	CV <sub>I</sub>	CN <sub>NL</sub>	RCV (%)
Pre-HD urea (mg/dL)	0.36	3.19	8.86%
Post-HD urea (mg/dL)	24.3	3.19	0.13%
Creat pre-HD (mg/dL)	10.8	4.1	0.37%
Creat post-HD (mg/dL)	12.9	4.1	0.31%
Calcium pre-HD (mg/dL)	NC	2.04	NC

\* Nc: not calculable. CV<sub>I</sub>: Intra-Individual Biological Variation.**Table 5.** Comparisons between previously published coefficients.

	CV <sub>I</sub> % (Ricos)	CV <sub>I</sub> % (Fraser)	CV <sub>I</sub> % (Study)	CV <sub>G</sub> % (Ricos)	CV <sub>G</sub> % (Fraser)	CV <sub>G</sub> % (Study)
Urea (mg/dL)	11.7	0.73	Pre 0.36	18.7	56.04	Pre 0.87
creatinine (mg/dL)	5.3	151.27	Pre 10.8	14.7	13722.93	Pre 24.23
Total calcium (mg/dL)	2.8	$2.27 \times 10^{-3}$	NC	2.5	$18.58 \times 10^{-3}$	Pre 7.31

\*Nc: no calculable. CV<sub>I</sub>: Intra-Individual Biological Variation. CV<sub>G</sub> Inter-Individual Biological Variation.**Table 6.** Comparisons between reference values of change between previously published studies.

	RCV (Ricos, et al 2014)	RCV (Study)
Urea (mg/dL)	9.5	Pre 8.89
Creatinine (mg/dL)	137	Pre 32.02
Total calcium (mg/dL)	2.77	NC

NC. not calculable

## Discussion

Biological variability criteria and Delta Check reduce diagnostic errors and speed up medical decision-making in patients in hemodialysis programs. As presented in this study, biological variability influences the monthly results. For the analysis of the results of this study, a two-factor ANOVA was used for the three analyses (total calcium, urea, and creatinine) with a total of 165 patients. These results are explained according to the metabolic or homeostatic regulation that occurs in the body after entering a renal function replacement program. This study demonstrates that patients with chronic renal failure generally have biological fluctuations around their homeostatic set points similar to those in healthy individuals.

None of the data were excluded because the results of the analyses of the quality control materials were satisfactory. In the case of calcium, the CV<sub>I</sub> data were not calculated, since

the estimation model of the intraindividual analytical variation, both in the average of the individual variance subtracting the analytical coefficient of variation obtained from the variance of the intermediate precision, although because the laboratory is within the quality standards, its variance exceeded the variance of the period. An essential piece of information is the relative variation coefficient (RCV), or percentage coefficient, the same that uses standardized criteria based on the intraindividual biological variability of the population studied and the analytical variability of each laboratory; the result must be less than one, in this study a CV<sub>R</sub> of urea was obtained 8.86% predialysis 0.13% postdialysis, and for creatinine, the prevalue is 0.37% and with a post value of 0.31. With calcium, the value was incalculable.

In studies carried out in other publications, the results differ for this study because they are heterogeneous populations; for example, studies carried out by Ricos [7] and Fraser [8] measured urea and creatinine in 17 patients





diagnosed with kidney failure, the sampling was eight determinations over 21 days and with calcium, 11 determinations were made in 9 subjects over 84 days as indicated in Table 4. Therefore, the study compared to these publications indicates the correlation, if any, between the severity of renal function impairment and the magnitude of intraindividual variation. Currently, there is no consensus indicating the time and interval of sampling or the number of subjects that should be taken into account for this type of study; it is suggested that these parameters are irrelevant to what these authors indicate. According to publications by Carmen Ricos, HRV values derived from BV data have been associated with the best tool available for the follow-up and monitoring of patients with chronic pathologies. The first to apply estimated BV data in patients with chronic pathologies were the authors Fraser and Philip; as indicated by the values in Tables 5 and 6, it has been shown that this approach allows the detection of changes in the patient's health status before that these have been clinically confirmed.

The intraindividual variation coefficient of predialysis urea was 0.36%, which implies a tiny variation in the urea averages throughout the study period. This means that the intake of urea precursors and proteins was constant over time in the study group. The intraindividual coefficient of variation of postdialysis urea was 24.3%, which implies that there are apparent differences in intraindividual treatments over time: Some months, the patients received more dialysis with an extracorporeal flow (EC) higher throughout the treatment and a lower decrease in postdialysis urea; in other months, the samples were taken at a lower dialysis flow (QB), which causes the differences between the months. Creatinine is related to muscle mass. It is observed that there is a decrease in predialysis serum creatinine at the time of the study, which implies a constant decrease in muscle mass, with a tendency to lose muscle as time goes by. The interindividual variation in total serum calcium was 7.31%, with the highest values in the first four months, possibly indicating a higher intake of calcium products during the first four months and then probably being abandoned by the patients.

## Conclusions

The intraindividual predialysis coefficient of variation for urea indicates minimal variation in urea averages throughout the study period associated with protein intake stability. The intraindividual coefficient of variation postdialysis of urea is related to the differences in the prescription of extracorporeal flow in each treatment. The variability of serum creatinine has implications for the constant loss of muscle mass over the study time. The interindividual variation in serum calcium was present at 7% and was associated with an increase in calcium

values in the first four months and a constant drop after this period, probably associated with a decrease in the intake of calcium products after the fourth month.

### Abbreviations

CVG: Biological Inter-Individual Variation.  
CVI: Intra-Individual Biological Variation.  
CCE: External Quality Control.  
CVA: Coefficient of Analytical Variation.  
CVB: Coefficients of Biological Variation.  
IDS: Index of standard deviation.  
CKF: Chronic Renal Failure.  
GFR: Glomerular filtration rate.  
RCV: relative coefficient of variation  
VA: Analytical Variation.  
VB: Biological Variation.  
VRC: Exchange Reference Variation.

### Supplementary information

Supplementary materials have not been declared.

### Acknowledgments

The staff and patients of the Clinef - Norte Dialysis Center Clinic, who collaborated in this study, are acknowledged.

### Author contributions

Cristina Paola Vernaza Guerra: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Methodology, Project Management, Resources, Software, Writing – original draft.

Lourdes Alicia Pazmiño Martínez: conceptualization, supervision, validation, visualization, and writing: review and editing.

All authors read and approved the final version of the manuscript.

### Financing

The authors provided research expenses. The laboratory studies and analyses were part of the usual institutional activity and were not expenses added to the institution or the patients.

### Availability of data or materials

The data sets generated and analyzed during the current study are not publicly available due to participant confidentiality but are available from the corresponding author upon reasonable academic request.

### Statements

#### Ethics committee approval and consent to participate

It was not required for a database study.

#### Consent to publication

This does not apply when images or photographs of the physical examination or radiography/tomography/MRI of patients are not published.

#### Conflicts of interest

The authors report having no conflicts of interest.

#### Author Information

Cristina Paola Vernaza Guerra: Clinical Biochemistry from the Central University of Ecuador (Quito, 2018). ORCID: <https://orcid.org/0000-0002-1575-856>



---

Lourdes Alicia Pazmiño Martínez: Doctor in biochemistry and pharmacy from the Central University of Ecuador (Quito, 2007). Master in Public Health from the Universidad San Francisco de Quito (Quito, 2012).

## References

1. Chung HJ, Song YK, Hong SK, Hwang SH, Seo HS, Whang DH, Nam MH, Lee DH. Implementation of variation biological-based analytical performance specifications in the laboratory: Stringent evaluation of Improvacutor blood collection tubes. *PLoS One*. 2017;12(12):e0189882. DOI: 10.1371/journal.pone.0189882. PMID: [29261759](#); PMID: PMC5738124.
2. Kinns H, Pitkin S, Housley D, Freedman DB. Internal quality control: best practice. *J Clin Pathol*. 2013;66(12):1027-32. DOI: 10.1136/jclinpath-2013-201661. Epub 2013 September 26. PMID: [24072731](#).
3. Haile B, Bikila D, Tewabe H, Wolde M. Preparation of In-House Quality Control Human Serum for Urea and its Use in Clinical Chemistry. *Clin Lab*. 2020;66 (3). DOI: 10.7754/Clin.Lab.2019.190704. PMID: [32162889](#).
4. Harris EK, Yasaka T. On the calculation of a "reference change" for comparing two consecutive measurements. *Clinic Chem*. 1983;29(1):25-30. PMID: [6848276](#).
5. Yépez E, Rosales W, Yansaguano M, Benítez R. Retinopathy in type 2 diabetic patients treated at Pablo Arturo Suarez Hospital from March 2010 to February 2011. [Specialty thesis in Internal Medicine]. Central University of Ecuador 2012; Institutional Repository: [dspace.use /T-UCF-0006-3](#)
6. Sinton TJ, Cowley DM, Bryant SJ. Reference intervals for calcium, phosphate, and alkaline phosphatase as derived based on multi-channel-analyzer profiles. *Clin Chem*. 1986;32(1 Pt 1):76-9. PMID: [3940739](#).
7. Ricós C, Alvarez V, Cava F, García -Lario JV, Hernández A, Jiménez CV, Minchinela J, Perich C, Simón M. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest*. 1999;59(7):491-500. DOI: 10.1080/00365519950185229. PMID: [10667686](#).
8. Fraser C, Singer R. Better laboratory evaluations of instruments and kits are needed. *Clinical Chemistry* 1985;31(5):667..

DOI: Digital Object Identifier PMID: PubMed Identifier SU: Short URL

---

## Editor's note

The REV SEN remains neutral concerning jurisdictional claims on published maps and institutional affiliations.

---