American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Validation of Irma Cgea/crenk One Method of Evaluation of the Vitamin D at the Regional Center of Nuclear Study Laboratory of Kinshasa

Ingala P[a*], Lepira F[b], Mputu L[c]

[a,c]*Department of Obstetrics and Gynecology, University of Kinshasa Hospital, Kinshasa, 00243, Democratic Republic of Congo*

[b]*Department of Internal Medicine, University of Kinshasa, Kinshasa, 00243, Democratic Republic of Congo*

Abstract

The aim of the present study was to validate the method IRMA CGEA/CRENK for the evaluation of the vitamin D status by comparison to the commercial method DIASORIN. A blood sample was obtained from 30 volunteers with a good health state in general population (11 women and 19 men) to determine serum 25(OH) D, concentrations by all those methods. Using local laboratory cutoffs, vitamin D insufficiency and deficiency was defined as 25(OH) D values of 4-14 ng/ml and < 4 ng/ml, respectively; vitamin D sufficiency as ≥15ng/ml [23]. Statistical analyses were performed using Excel 12.0 and SPSS 21 statistical software's. Mean and standard deviation (SD), were used to evaluate the mean of vitamin D of the two methods. Student t TEST was used to compare mean of the two methods. P value ≤ 0.05 defined the level of statistical significance. PEARSON correlation coefficient (**r**) was performed to evaluate the correlation between the two methods. An r=0 means the missing of correlation: r<0 means a negative correlation; r=1 means a positive and perfect correlation; 0, 75<r<1 means a positive and strong correlation. The concordance between IRMA CGEA/CRENK and the commercial method DIASORIN has been definite as the missing of statistic difference between the means of vitamin D with the two methods and a positive correlation between the levels of vitamin D of the two methods. The coefficient kappa was calculated for avoiding the rate of random in the concordance. The variations of the coefficient Kappa is ±1. K= -1 means total discordance. K=+1 means an absolute concordance. K near 0 means a mean concordance explain by random. K>0, 8 (80%) is the limit of the better concordance. No statistic difference was observed between the means of vitamin D of the method IRMA CGEA/CRENK and the commercial method DIASORIN (**p=0,330**). The coefficient of correlation was: **r = 0, 96**. The equation of correlation was: **y =1,429 + 1, 02 x** (y= IRMA CGEA/CRENK, x= DIASORIN Commercial). The coefficient kappa = **0, 85**.

Keys Words: Irma cgea/crenk-diasorin commercial-validation.

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* Corresponding author.

1. Introduction

The vitamin D can be evaluated by two methods: immunologic method including, radio immuno assay (RIA), enzymatic immuno assay (EIA) and chemilunescent immuno assay (CLIA) and the physic method of direct separation including chromatography and spectrometry [1]. IRMA CGEA/CRENK is one variety of the radio immuno assay used at the Regional Center of Nuclear Study laboratory of Kinshasa. At the first time we characterized the performance of that method [2]. Now we present the result of validation of that method according to the commercial method DIASORIN.

2. Material and methods

A blood sample was obtained from 30 volunteers (11 women and 19 men) to determine serum 25(OH) D, concentrations. All tubes were protected from light and the specimens were centrifuged at 2500 rpm for 10 minutes. The serum was separated and stored at -20°C until analyzed at the Regional Center of Nuclear (**CRENK**) Study of Kinshasa/University of Kinshasa. Serum 25(OH) D concentrations were measured using a local immunoradiometric assay (IRMA) method at the Regional Center of Nuclear Study laboratory of Kinshasa; this method has been described elsewhere [2]. Briefly, serum 25(OH) D was particularly captured by a homemade polyclonal antibody during specimen incubation in RIA tube during two hours at the room temperature (25°C); captured 25(OH) D then reacted with a commercial monoclonal anti 25(OH) D antibody labeled with Iodine-125. The same serums were also measured by using the commercial radio immuno assay DIASORIN. Briefly that method is a competitive one. The serum 25(OH) D make challenge against a solution of the radioactive vitamin D antigen (tracer) for the fixation to the monoclonal antibody anti-vitamin D. The level of vitamin is inversely proportional to the radio activities captured. Using local laboratory cutoffs, vitamin D insufficiency (**H**) and deficiency (**h**) was defined as 25(OH)D values of 4-14 ng/ml and < 4 ng/ml, respectively; vitamin D sufficiency (**N**) as ≥15ng/ml [23].

Statistical analyses were performed using Excel 12.0 and SPSS 21 statistical software's. Mean and standard deviation (SD), were used to evaluate the mean of vitamin D with the two methods. Student t TEST was used to compare mean of the two methods. P value ≤ 0.05 defined the level of statistical significance. PEARSON correlation coefficient (**r**) was performed to evaluate the correlation between the two methods. . An r=0 means the missing of correlation: r<0 means a negative correlation; r=1 means a positive and perfect correlation; 0, 75<r<1 means a positive and strong correlation. The concordance between the method IRMA used at the laboratory and the method DIASORIN is definite as the missing of statistic difference between the means of vitamin D with the two methods and a positive correlation between the levels of vitamin D with the two methods. The coefficient kappa (K) was calculated for avoiding the rate of random in the result of concordance. The variations of the coefficient Kappa is ±1. K= -1 means total discordance. K=+1 means an absolute concordance. K near 0 means a mean concordance explain by random. K>0, 8 (80%) is the limit of the better concordance.

The study received the clearance of the Ethical Committee of Kinshasa School of Public Health/University of Kinshasa (ESP/CE/N°028/2013).

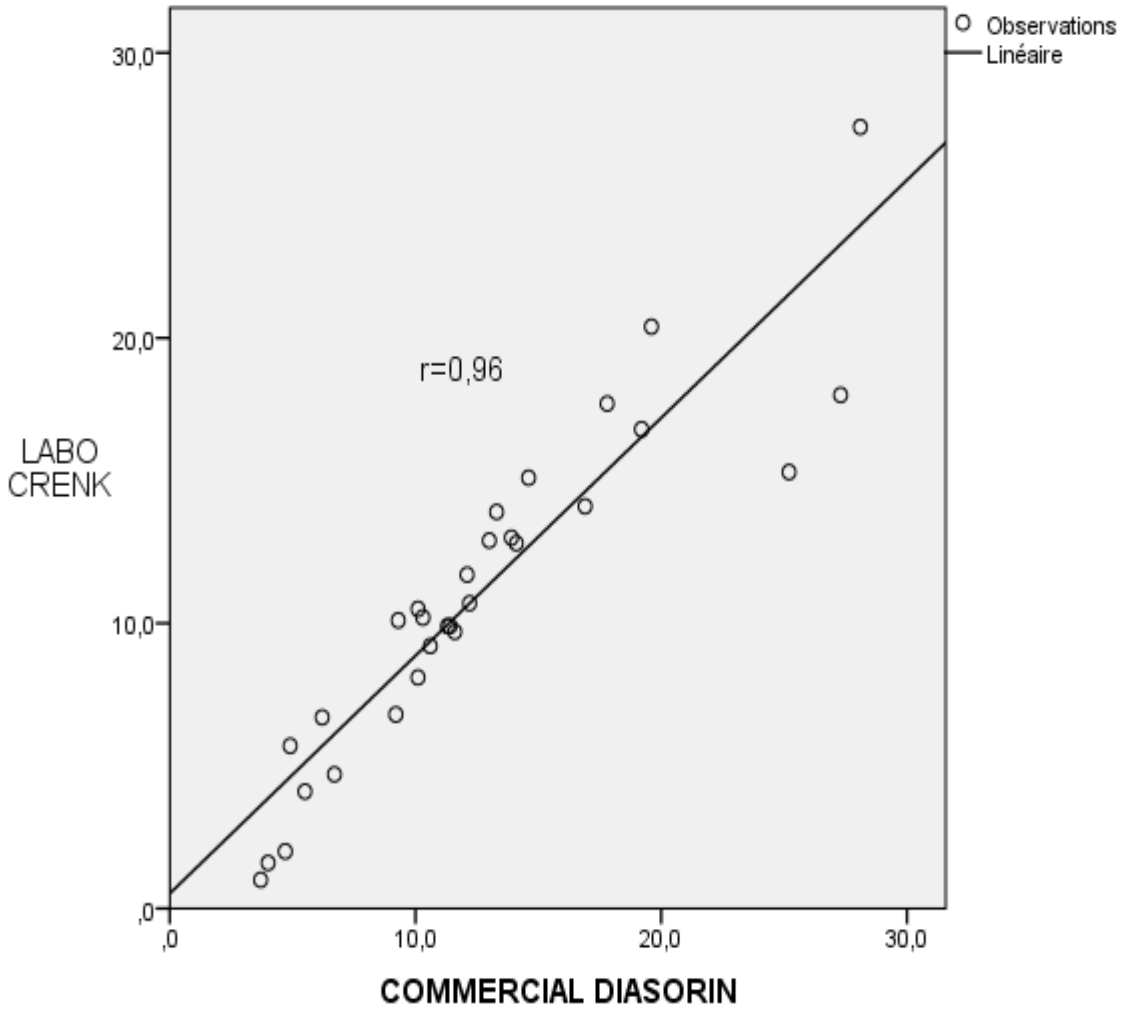
3. Results

Table 1: comparison of the means of vitamin d of the methods irma cgea/crenk vs diasorin commercial

CGEA (OH) D IRMA	DIASORIN
9,9	11,3
13	13,9
9,7	11,6
9,9	11,4
16,8	19,2
27,4	28,1
17,7	17,8
8,1	10,1
14,1	16,9
4,7	6,7
1	3,7
6,8	9,2
11,7	12,2
10,7	12,2
10,2	13
12,9	13
1,6	4
5,7	4,9
9,2	10,6
12,8	14,1
13,9	13,3
10,5	10,1
20,4	19,6
10,1	9,3
4,1	5,5
18	27,3
6,7	6,2
15,1	14,6
15,3	25,2
2	4,7
Mean ±SD=11,0±5,8	Mean ± SD=12±6,4
P=0,330	

The table one shows that there is no difference between the means of vitamin D of the method IRMA

CGEA/CRENK (OH) D IRMA and the commercial method DIASORIN : **p value =0,330**.



Graphic 1: coefficient of correlation between the methodscgea/crent vs diasorin commercial and equation of regression

Y =1,429 + 1,02 X **Y= CRENK** **X= Commercial Diasorin**

The graphic bellow shows that it exist high positive correlation between the method IRMA and the method DIASORIN (**r= 0, 96**). The equation of regression is **Y=1,429+1,02X.**

According to the table 2 the following results have been observed: N=8, H=19, h=3 with IRMA.N=9, H=20, N=9 with DIASORIN. **28** cases of concordance (**n**) and **2** cases of discordance (**m**) between IRMA and DIASORIN among 30 comparisons (**N**).

Table 2: concordance between the methods irma cgea/crenk and diasorin commercial

IRMA		DIASORIN		GLOBAL APPRECIATION	
Value	Appreciation	Value	Appreciation	Concordance (**c**)	Discordance (**d**)
9,9	H	11,3	H	C	
13	H	13,9	H	C	
9,7	H	11,6	H	C	
9,9	H	11,4	H	C	
16,8	N	19,2	N	C	
27,4	N	28,1	N	C	
17,7	N	17,8	N	C	
8,1	H	10,1	H	C	
14,1	N	16,9	N	C	
4,7	H	6,7	H	C	
1	H	3,7	H	C	
6,8	H	9,2	H	C	
11,7	H	12,2	H	C	
10,7	H	12,2	H	C	
10,2	H	13	H	C	
12,9	H	13	H	C	
1,6	H	4	H		D
5,7	H	4,9	H	C	
9,2	H	10,6	H	C	
12,8	H	14,1	H	C	
13,9	H	13,3	H	C	
10,5	H	10,1	H	C	
20,4	N	19,6	N	C	
10,1	H	9,3	H	C	
4,1	H	5,5	H	C	
18	N	27,3	N	C	
6,7	H	6,2	H	C	
15,1	N	14,6	N	C	
15,3	N	25,2	N	C	
2	H	4,7	H		D
Total				28	2

Legend: **H**= high level of vitamin D over the normal ranch **N**= normal level of vitamin D **h**=vitamin D inferior to the normal ranch **C**= concordance **D**=discordance.

Table 3: concordance between the methods irma cgea/crenk vs diasorin in the ranches of the vitamin d

DIASORIN IRMA	N	H	H	TOTAL
N	8	0	0	8 (P1)
H	0	19	0	19 (P2)
H	0	2	1	3 (P3)
TOTAL	8 (T1)	21 (T2)	1(T3)	30(N)

Legend: **H**= high level of vitamin D over the normal ranch **N**= normal level of vitamin D **h**=vitamin D inferior to the normal ranch **P1,P2, P3**= horizontal position of the value **T1,T2,T3**=vertical position of the value

According to the table 2, **t**he table 3 show the following results: **T1P1=8×8= 64; T2P2=21×19=399; T3P3 =1×3=3.**

4. Coefficient of concordance and coefficient kappa

According to the results in the table 2 and the table 3 the following evaluations have been obtains:

- Coefficient of concordance (Cc) = n / N = 28:30= 0,93
- Coefficient of concordance waited (Ca) = T_1P_1 +T_2P_2+T_3P_3+…….TnPn / N^2 = 8×8+ 21×19+1×3/30^2=0,51
- Coefficient Kappa (K)= Cc- Ca / 1-Ca =0,93-0,51 / 1-0,51 =85%

5. Discussion

Several methods are applicated to determine the status of the vitamin D [1]. According to De la Hunty, the weakness of all those methods are their insufficiency of sensitivity, the matrix effect and the absence of standardization [3, 4, 5] that why considering the Joint Committee for Traceability in Laboratory Medicine (JCTLM) the gold standard method for evaluation of the status of the vitamin D doesn't exist up to now [6,7,8].

However for the National Institute of Standard and Technology (NIST) the association of chromatography HPLC with mass Spectrometry (LCMS) represents actually the best method to evaluate the status of the vitamin D [9]. But that method is very expensive and request a high performants material and a personal especially trained. That why that method is not accessible to number countries and consequently the status of the vitamin D depend always on a laboratory to another.

IRMA CGEA/CRENK is the method of the diagnosis of the status of the vitamin D at the laboratory of the

Regional Center of Nuclear Study laboratory of Kinshasa. The interesting advantage of the kind of method is the possibility of each laboratory to produce itself the poly clonal anti-body anti-vitamin D and its high sensitivity. That why she is particularly indicate for the counties of weak resources.

According to the resolutions of the workshop on the quality and the chemistry organized in Montpellier in FRANCE in 2010 by IBMM (Institute of biomolecules Max Mousseron) [10], specificity, linearity, ranch of application, ranch of detection, ranch of quantification, stability and strongness are criteria to consider for the validation of a biologic method. However there is not only one protocol of that validation.

The most important recommendation request that the method must be precise, linear in its ranch of linearity, specific, sensitive, exact et concordance with an equivalent commercial method. Such are the criteria we have taken account to determine the performances of the method IRMA CGEA/CRENK [2].

For the validation of the method IRMA CGEA/CRENK, the reference method was the commercial method DIASORIN. We considered two criteria: the significance of the means of the vitamin D and the correlation between those methods. The coefficient Kappa serves to calculate the rate of random in the concordance. There was not a statistic difference between the means of vitamin D (p=0,330). A high correlation was found (r=0, 96). The coefficient Kappa was important: (K=0, 85).

For others equivalent studies, the reference method has been the association HLPC and Mass Spectrometry (LCMS) for the validation of the method Diasorin RIA. It was the case of Mansell and his colleagues who observed r=0, 91 [11]; Saenger and his colleagues who found r= 0, 97 [12] and Chen and his colleagues who reported r=0, 96[13].

A good correlation was also found between LCMS and the method IDS EIA at the concentrations less than 125 nmol/l. [14].

6. Conclusion

The present study show that the IRMA CGEA/CRENK has a positive and strong correlation with the commercial method DIASORIN and appear as a good alternative for the evaluation of the status of the vitamin D especially for a country of less resources.

Acknowledgement

The authors gratefully thank, medical team of University clinics of Kinshasa, Saint Joseph Hospital and Edith medical Center for their invaluable help for the collection data of the study.

References

[1]. HAS (Haute Autorité de Santé).Note de cadrage.2013 Jan.www.has-sante.fr

[2]. Tshiband A, Mputu L,Tozin R, Ingala , Kiampa Solid-Phase Immuno Radio Metric Assay(IRMA) of

25-hydroxy vitamin d and displacement from serum binding proteins for Resource –Limited Settings. Journal of Biomedical Engineering and Medical Devices.2015; 1: 1..

[3]. De la Hunty A., Wallace AM., Gibson S., Vijakalmen H., Lamberg-Allrdt C., Achwell., UK Food Standards Agency Workshop Consensus Report; the choice of method for measuring 25-hydroxyvitamin D to estimate vitamin D status for the UK National Diet and Nutrition Survey.Br J Nutr 2010; 104(4): 812-9.

[4]. Wallace AM., Gibson S., de la HA., Lamberg-Allardt C., Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory : current procedures, performance characteristics and limitations. Steroids 2010; 27 (2): 477-88.

[5]. Ingrand J. La spectrométrie de masse et ses principales applications en biologie médicale. Immuno-analyse et biologie spécialisée.2012; 27(7):47-53

[6].Institute of Medicine. Ross AC., Taylor CL., Yaktine AL., Del Valle HB. Dietary reference intakes for calcium and vitamin D. Washington; National Academics Press; 2011. http://www.ncbi.nih.gov/books/NBK56070/pdf/TOC.pdf

[7]. Groupe de Recherche et d'information sur les Ostéoporoses.BenhamouC-L., Souberbielle J-C., Cortet B., Fardellone P., Gauvain J-B et al. La vitamine D chez l'adulte : recommandations du GRIO. Presse Med 2011 ; 40 : 673-82.

[8]. Tai SS., Bedner M., Phiney KW. Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitaminD3 and 25-hydroxyvitamin D2 in human serum using isotope dilution liquid chromatography-tandem mass spectrometry. Anal Chem 2010; 82(5): 1942-8.

[9]. National Institute of Standard and Technology. Standard Reference Material 972.VitaminD in Human Serum. Gaithersburg: NIST; 2009.

[10].Marie-Dominique Blanchin. Validation des méthodes d'analyse. Journées Qualité et Chimie 2010.Une démarche qualité au service de la chimie. Autrans-14 octobre 2010. Mdb-14 octobre 2010.

[11]. Maunsell Z., Wright DJ., Rainbow SJ. Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxyvitamin D2 and D3. Clin Chem. 2005; 51:1683-90.

[12]. Saenger AK., Laha TJ., Brenner DE., Sadrzadeh SMH. Quantification of serum 25-hydroxyvitamin D2 and D3 using HLPC-tandem mass spectrometry and examination of reference intervals for diagnosis of vitamin D deficiency. Am J Clin Pathol 2006; 125: 914-20.

[13]. Chen H., McCoy LF., Schleider RL., Pfeiffer CM. Measurement of 25-hydroxyvitamin D3 25OHD3

and 25-hyroxyvitamin D2 25OHD2 in human serum using liquid chromatography-tandem mass spectrometry and its comparison to a radioimmunoassay method. Clin Chim Acta 2008; 391:6-12.

[14]. Knox S., Harris J., Calton L., Wallace AM. A simple automated solid-phase extraction procedure for measurement of 25-hydroxyvitamin D3 and D2 by liquid chromatography- tandem mass spectrometry. Anal Clin Biochem 2009; 46: 226-30.

7. Appendix: author's contributions

PIERRE INGALA, participate in protocol elaboration, data collection and analysis and draft the manuscript.

FRANCOIS LEPIRA, participate in protocol elaboration, conception and data analysis reviewed the manuscript.

SERGE MUHINDO, participate in protocol elaboration, data collection and analysis and draft the manuscript.

ARSNE MPUTU, conceived the study, participate in data analysis and reviewed manuscript.