



30. Simpozij HDMBLM-a

# ANALITIČKI IZAZOVI U ODREĐIVANJU VITAMINA D

Klinički zavod za kemiju  
KBC Sestre milosrdnice, Zagreb

# Zašto 25(OH)D kao biomarker???

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- **Funkcionalni indikator vit D statusa**
- Procjena statusa nutricije



Metabolit	Koncentracija	% sl. oblika	Poluvijek
Vitamin D	<0.5-52 nmol/L	—	1-2 dana
25-hidroksivitamin D	25-162 nmol/L	0.03	2-3 tjedna
1,25-dihidroksivitamin D	36-144 pmol/L	0.4	4-6 sati
24,25-dihidroksivitamin D	2-7.2 nmol/L	?	NA

# Klinički značaj

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## ↓ 25-(OH)D

- Nedovoljno izlaganje sunčevoj svjetlosti
- Deficijentna prehrana
- Malapsorpcijski sindromi
- Teška jetrena bolest
- Primjena antikonvulziva
- Nefrotski sindrom

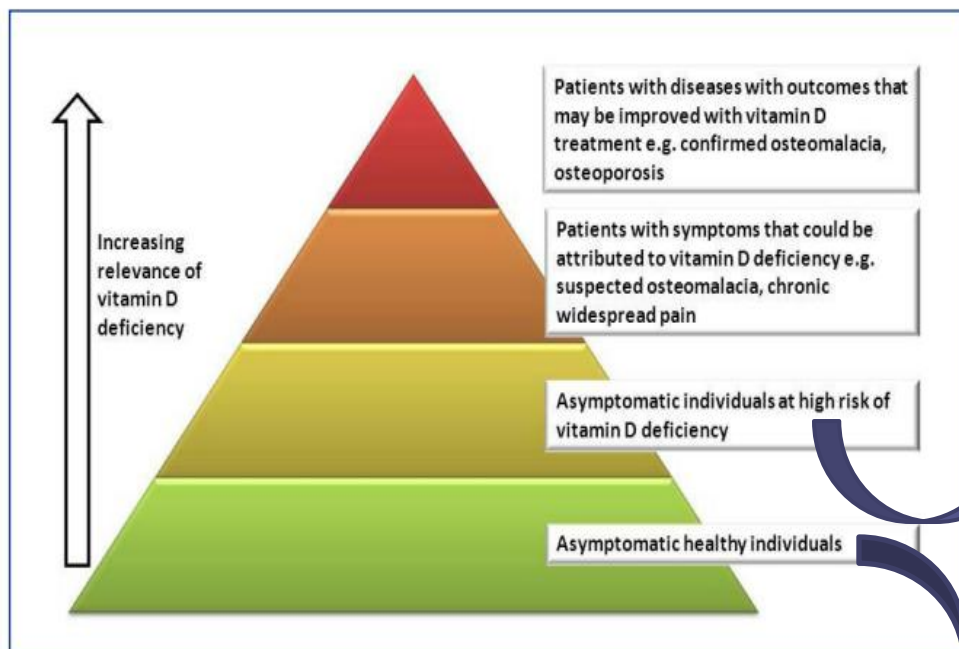
## ↑ 25-(OH)D

- Intoksikacija uslijed ingestije Vit D ili 25-(OH)D

# Koga rutinski testirati?

## Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management

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**Figure 3** Schematic representation that helps to define broad groups for clinical consideration and decision making.

### Asymptomatic healthy individuals

The use of serum 25OHD measurements in asymptomatic healthy individuals and the correction of deficiency to reduce the incidence of the diseases putatively associated with vitamin D deficiency have never been studied. This form of population screening has not been carried out and would not fulfil recognised criteria for screening <sup>48</sup>. Although vitamin D deficiency is highly prevalent, universal screening of asymptomatic populations is not recommended.

### Asymptomatic individuals at higher risk of vitamin D deficiency

There are a number of risk factors in asymptomatic individuals that predispose to lower levels of 25OHD. These individuals are more likely to be vitamin D-deficient and current UK guidance from the Department of Health recommends that these individuals have a higher intake of vitamin D (see box below).

### Recommendation:

Do not routinely test 25OHD levels in these groups.

### Department of Health Guidance<sup>iii</sup>

#### Adult groups at risk of vitamin D deficiency:

- all pregnant and breastfeeding women, especially teenagers and young women
- older people, aged 65 years and over
- people who have low or no exposure to the sun, for example those who cover their skin for cultural reasons, who are housebound or who are confined indoors for long periods
- people who have darker skin, for example people of African, African-Caribbean or South Asian origin, because their bodies are not able to make as much vitamin D.

Nevertheless, routine 25OHD testing may be unnecessary in patients with osteoporosis or fragility fracture, where a decision has been made to co-prescribe vitamin D supplementation with an oral antiresorptive treatment.

# Indikacije u HR

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## SMJERNICE ZA PREVENCIJU, PREPOZNAVANJE I LIJEČENJE NEDOSTATKA VITAMINA D U ODRASLIH\*

### GUIDELINES FOR THE PREVENTION, DETECTION AND THERAPY OF VITAMIN D DEFICIENCY IN ADULTS\*

DARIJA VRANEŠIĆ BENDER, ZLATKO GILJEVIĆ, VESNA KUŠEC,  
NADICA LAKTAŠIĆ ŽERJAVIĆ, MARIJA BOŠNJAK PAŠIĆ, EDUARD VRDOLJAK,  
DINA LJUBAS KELEČIĆ, ŽELJKO REINER, BRANIMIR ANIĆ, ŽELJKO KRZNARIĆ\*\*



1. NE preporučuje se mjerenje u krvi zdravih osoba bez rizičnih čimbenika!
2. Ponovljeno određivanje 3 mjeseca nakon uvođenja liječenja pripravcima!
3. Kontrolno mjerenje važno pri liječenju visokim dozama vitamina, na terapiji bifosfonatima i u osoba s rizikom od prijeloma kuka
4. U rizičnih skupina mjerenje na kraju zimskog razdoblja

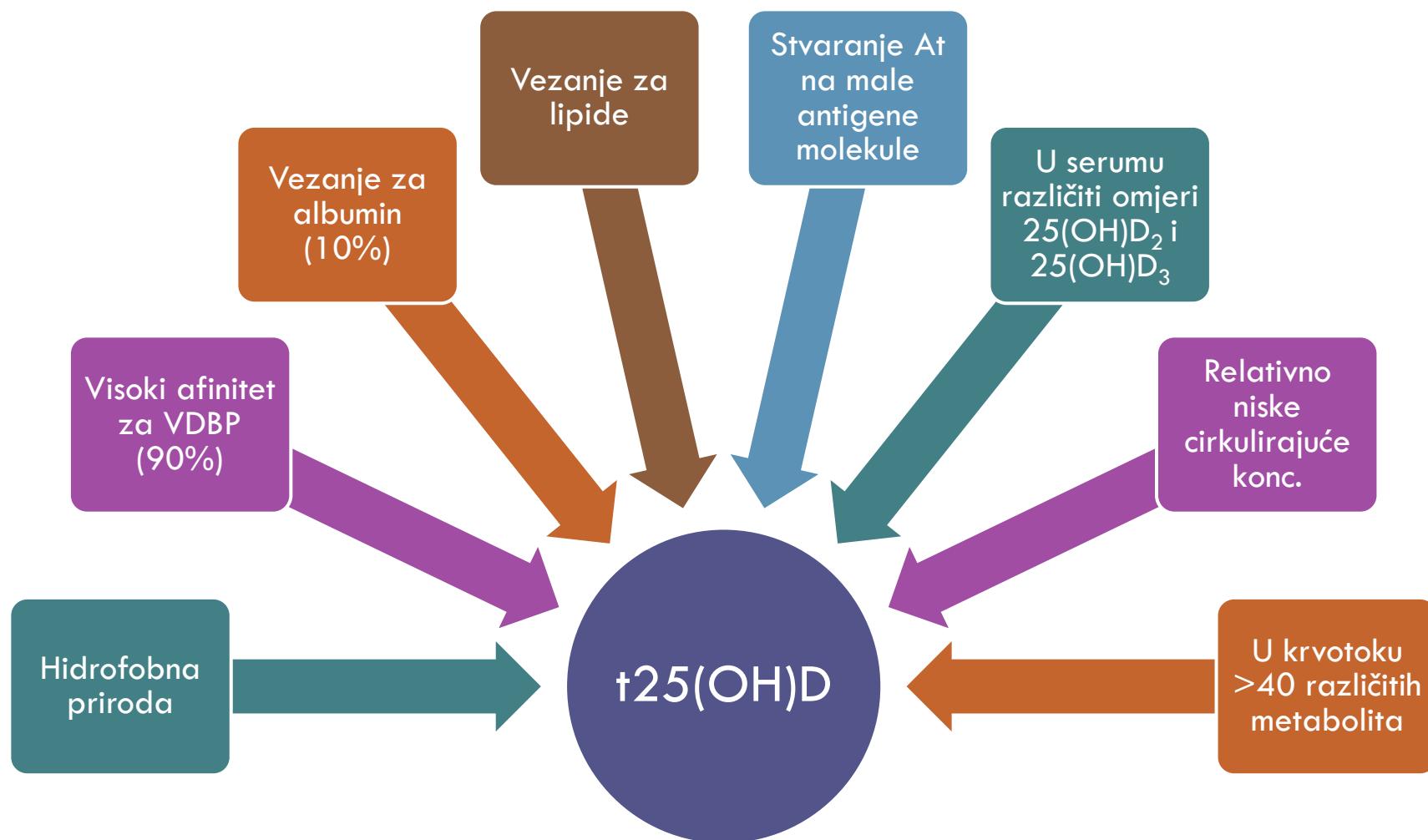
Tablica 2. Čimbenici rizika od razvoja nedostatka vitamina D u odraslih  
(prilagođeno prema <sup>21</sup> i <sup>13</sup>)

Table 2. Risk factors for vitamin D deficiency in adult population (adapted by <sup>21</sup> i <sup>13</sup>)

- Znakovi, simptomi i/ili planirana terapija rahitisa, osteoporoze ili osteomalacije / Signs, symptoms or planned therapy of rickets, osteoporosis or osteomalacia
- Povišena aktivnost alkalne fosfataze uz normalne vrijednosti krvnih pokazatelja funkcije jetre / High level of alkaline phosphatase with normal values of serum liver parameters
- Hiperparatiroidizam, hipokalcemija ili hiperkalcemija, hipofosfatemija / Hyperparathyroidism, hypo- or hypercalcemia, hypophosphatemia
- Malapsorpcija (npr. cistična fibroza, sindrom kratkog crijeva, upalna bolest crijeva, neliječena celijakija, barijatrijski zahvat, radijacijski enteritis) / Malabsorption (e.g. cystic fibrosis, short bowel syndrome, inflammatory bowel disease, untreated celiac disease, bariatric surgery, radiation enteritis)
- Tamna put ili dugotrajno izbjegavanje/nemogućnost izlaganja sunčevu svjetlu (zbog kulturoloških, medicinskih ili profesionalnih razloga) / Dark skin, decreased sun exposure (due to cultural, medical or professional reasons)
- Učestala uporaba zaštitnih sredstava za sunce / Heavy use of sunscreens
- Primjena lijekova koji utječu na sniženje koncentracije 25-OH D u krvi (poglavito glukokortikoidi, antiepileptici i antimikotici) / Use of medications (glucocorticoids, antiepileptic medications, antifungals)
- Bolesnici s kroničnim bubrežnim bolestima (KK < 65 ml/min) i bolesnici s bubrežnim presatkom / Patients with chronic kidney disease (CC < 65 ml/min) and patients with kidney transplant
- Zatajenje jetrene funkcije / Liver failure
- Trudnoća i dojenje / Pregnancy and lactation
- Starija životna dob (> 50 god.), osobito s anamnestičkim podatkom padova i prijeloma / Older age (> 50 years), especially with history of falls and fractures
- Pretilost (BMI > 30) / Obesity (BMI > 30)
- Granulomatozne bolesti (sarkoidoza, tuberkuloza, histoplazmoza, kokcidioza, berilioza) / Granuloma-forming disorders (sarcoidosis, tuberculosis, histoplasmosis, coccidiomycosis, berylliosis)
- Neke vrste limfoma / Some lymphomas

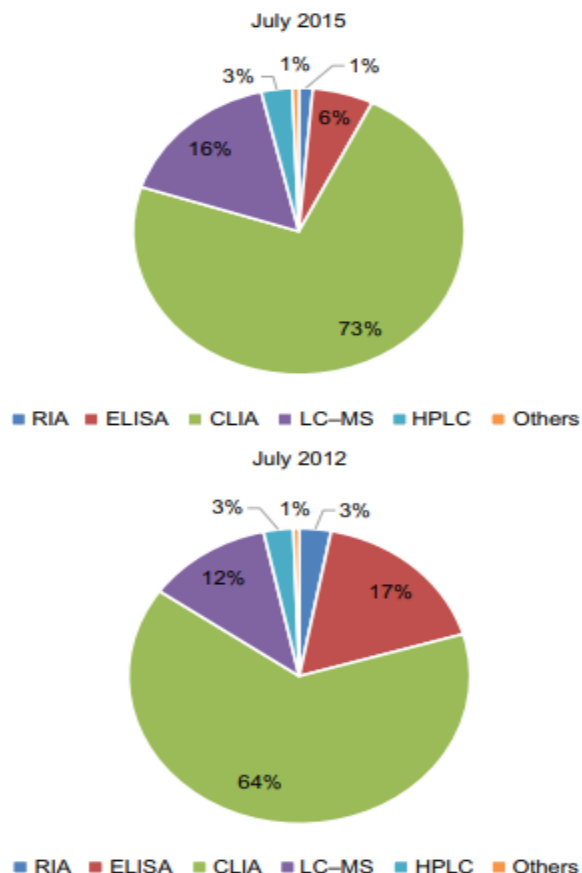
# “Težak analit”

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# Metodologija

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**Fig. 13** DEQAS July 2015: Distribution of 25OH Vitamin D methods participating to the DEQAS program in July 2015 and in July 2012.

Heureux N. Vitamin D Testing – Where Are We and What Is on the Horizon? *Adv Clin Chem.* 2017;78:59-101.doi: 10.1016/bs.acc.2016.07.002.

Table 1

Currently used vitamin D methods

Method	Proportion, %
Radio immunoassay	2
Manual immunoassay	9
Automated immunoassay	69
LC-MS/MS	15
Other	5

LC-MS/MS - liquid chromatography-tandem mass spectrometry.

Table 1. Currently used vitamin D methods

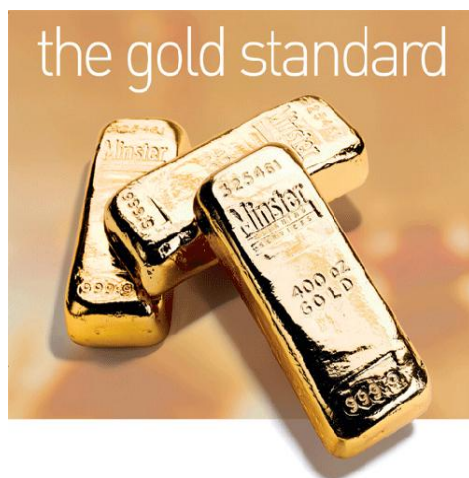
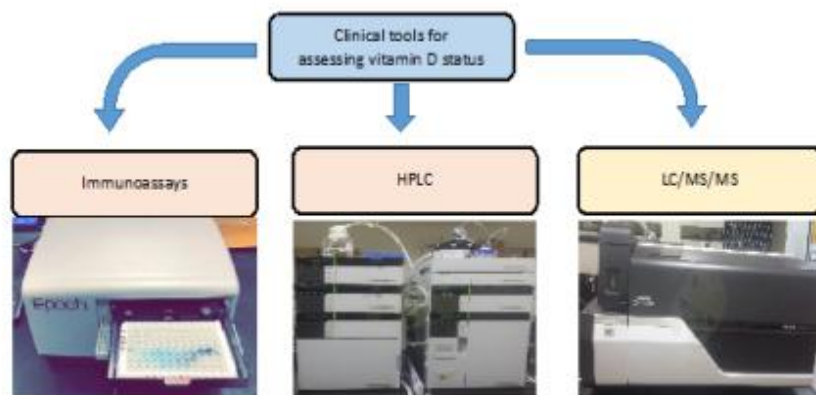
Ferrari D, Lombardi G, B Giuseppe. Concerning the vitamin D reference range: pre-analytical and analytical variability of vitamin D measurement. *Biochem Med.* 2017;27:1-14.doi: 10.11613/BM.2017.030501.



# Analitička varijabilnost



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Različite metode  
ekstrakcije i  
deproteinizacije

Križna reaktivnost  
At s epimerima  
i/ili ostalim  
metabolitima

Čimbenici

Prisutnost  
izobarnih spojeva

Interferencije  
matriksa



# Prednosti i nedostaci u kliničkoj praksi

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Table 2. Some characteristics of the assays for 25-OH-vitamin D analysis in clinical practice		
Method	Advantages	Disadvantages
Manual and automated IAs		
RIA after extraction	Extraction step minimizes matrix effects (RIA)	Time-consuming and radioactive residues (RIA)
EIA	Technically simple	Susceptible to matrix effects (EIA, automated IAs)
Automated IAs:	Relatively low-cost (RIA, EIA)	Lot-to-lot variability
[Liaison (Diasorin); iSYS, Architect (Abbott), Elecsys (Roche), Advia Centaur (Siemens)]	High throughput (Automated)	Variable recovery/ reactivity for 25-OH-D <sub>2</sub> , 3-epi-25-OH-D and 24, 25(OH) <sub>2</sub> D
Chromatographic methods		
HPLC	Extraction step minimizes matrix effects (interferences)	High-cost equipments (but low costs of reagents)
and	Simultaneous determination of 25-OH-D <sub>3</sub> , 25-OH-D <sub>2</sub> , 3-epi-25-OH-D <sub>3</sub> and 24, 25(OH) <sub>2</sub> D is possible	Expertise required
	High precision and accuracy	Higher sample volumes
	Direct control of the method (Standardization)	Labour-intensive/Time-consuming/Lower throughput (automatization is possible)
	Adjustable to reference method	
LC /MS /MS	Simultaneous determination of other serum components (vitamin A, E, coenzyme Q10, carotenoids)	

EIA, enzyme immunoassay; HPLC, high-performance liquid chromatography; IA, immunoassay; LC, liquid chromatography; MS, mass spectrometry; RIA, radioimmunoassay.

Granado Lorenzo F, Blanco-Navarro I, Pérez-Sacristán B. Critical evaluation of assays for vitamin D status. *Curr Opin Clin Nutr Metab Care*. 2013;16:734-40.doi: 10.1097/MCO.0b013e328364ca96.

# 1. Metode temeljene na kromatografskom razdvajanju

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Odvojena kvantifikacija  
 $25(\text{OH})\text{D}_2$  i  $25(\text{OH})\text{D}_3$

Implementacija izotopnih  
standarda vitamina –  
interni standardi

↑ osjetljivost ( $<2.5 \text{ nmol/L}$ ),  
točnost i ponovljivost (CV  
2 – 7-8%)

*In-house* metode i  
pročišćavanje seruma  
prije analize

Ko-eluirani  
spojevi u  
ekstraktu uzorka  
– ionska  
supresija

C3-epi-  
 $25(\text{OH})\text{D}$  i 7-  
 $\alpha$ -hidroksi-4-  
kolesten-3-  
on



FOTOREARCH®

## 2. Imunometode temeljene na vezanju At i proteina



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Specifičnost At i neekvimolarni odgovor na 25(OH)D<sub>2</sub> i 25(OH)D<sub>3</sub>

Križna reaktivnost s ostalim metabolitima (24,25(OH)D<sub>3</sub> do 13nmol/L)

Razlike u standardizaciji

Vezanje 25(OH)D za VDBP – lažno ↓ konc.

Interferencije matriksa

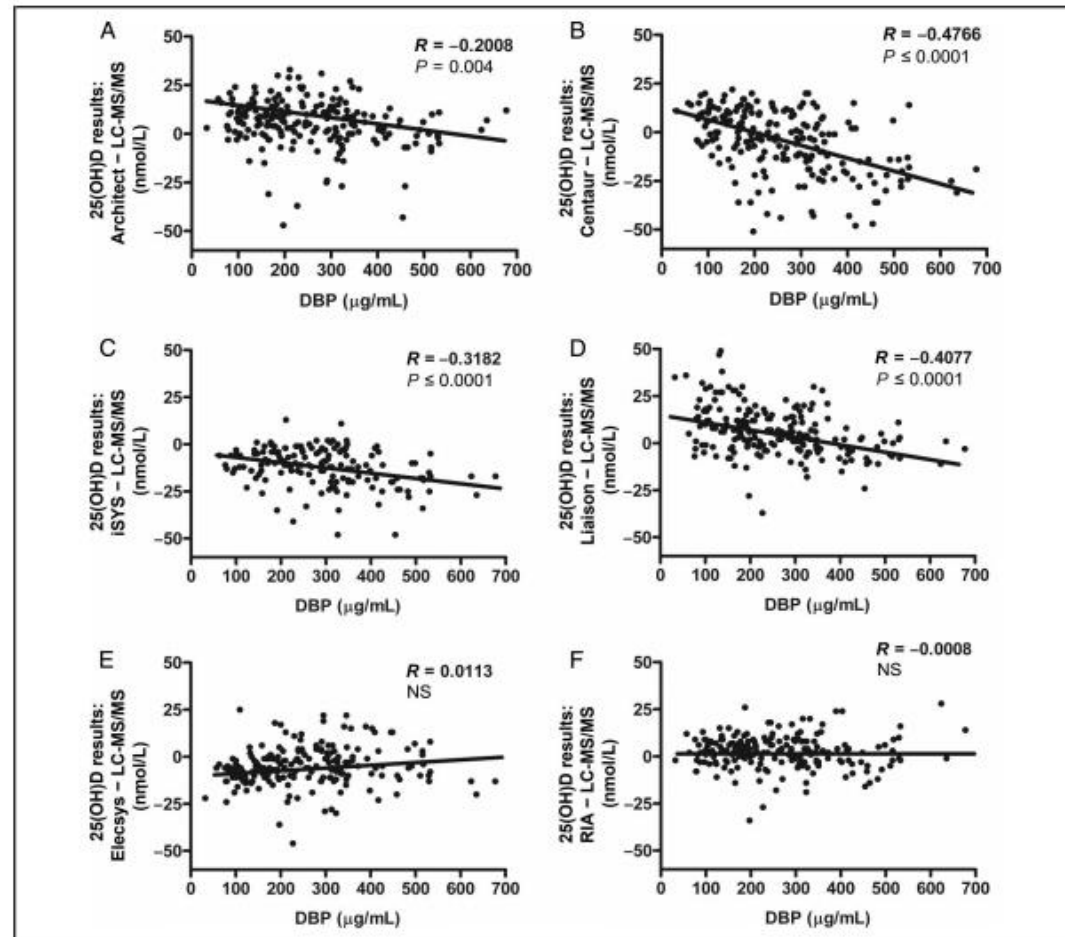


Fig 3. Correlation of the DBP concentration and the difference between the results of each of 6 routine 25(OH)D assays and the results of the ID-XLC-MS/MS assay.



# Klinički zavod za kemiju

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Presječno istraživanje  
Siječanj – veljača 2019.  
N = 200 (ambulantni pacijenti)  
Medijan dobi = 62 god (9-83)

**Table 2.** General information about participants.

Variable	N = 200
Males, N (%)	39 (19.5)
Vitamin D supplements intake, N (%)	87 (43.5)
Form of supplement, N (%)	
drops	65 (74.7)
ampoules	18 (20.7)
pills	4 (4.6)
Ordering physician, N (%)	
dermatologist	16 (8)
endocrinologist	32 (16)
neurologist	25 (12.5)
other	34 (17)
general practitioner	42 (21)
rheumatologist/physiatrist	51 (25.5)
Vitamin D (nmol/L)	
Architect	49 (29-70)
Mindray	52 (38-70)

Quantitative variables are presented as median (interquartile range).



# Bland-Altmanova analiza

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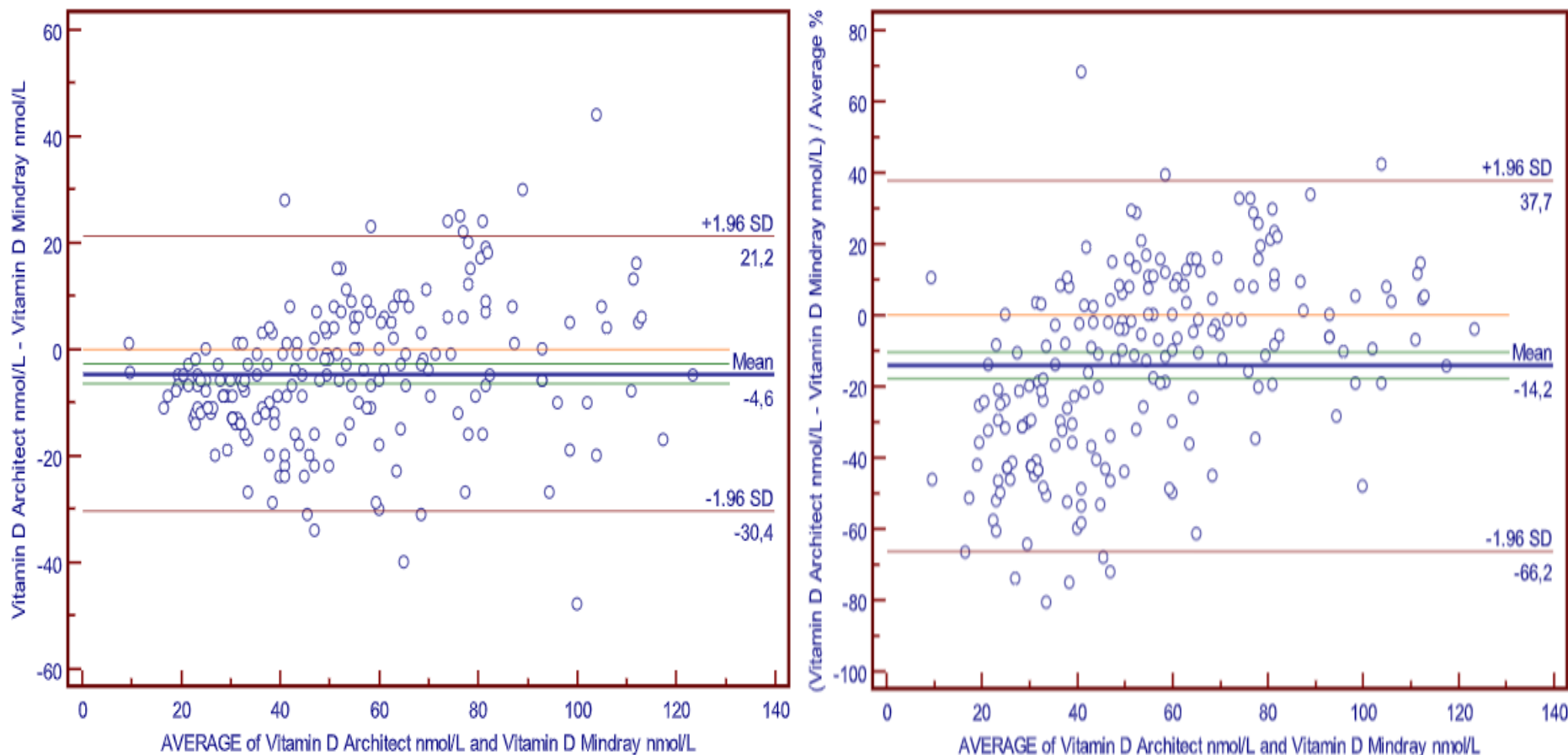


Figure 1. Comparison of 25(OH)D measured with Abbott Architect i2000 SR and Mindray CL-1000i in all participants. A mean difference of 4.6 nmol/L (Figure 1A) or 14.2% (Figure 1B) in 25(OH)D concentration was statistically significant. Solid line (mean) – mean difference. Dashed lines (SD) – standard deviation. Dotted line - 95% CI of mean of difference



**Table 3.** Agreement between Abbott and Mindray assay in assessment vitamin D status.

Vitamin D status	All		Supplement intake		No supplement intake	
	Abbott Architect i2000SR	<u>Mindray CL-1000i</u>	Abbott Architect i2000SR	<u>Mindray CL-1000i</u>	Abbott Architect i2000SR	<u>Mindray CL-1000i</u>
<b>deficiency (&lt;25 <u>nmol/L</u>)</b>	35 (17.5%)	10 (5.0%)	4 (4.6%)	0 (0.0%)	31 (27.4%)	10 (8.8%)
<b>insufficiency (25-75 <u>nmol/L</u>)</b>	126 (63.0%)	157 (78.5%)	48 (55.2%)	61 (70.1%)	78 (69.0%)	96 (85.0%)
<b>sufficiency (&gt;75 <u>nmol/L</u>)</b>	39 (19.5%)	33 (16.5%)	35 (40.2%)	26 (29.9%)	4 (3.5%)	7 (6.2%)
Total	200		87		113	
<b>Weighted kappa (95% confidence interval)</b>	0.55 (0.44 to 0.66)		0.66 (0.52 to 0.80)		0.35 (0.19 to 0.51)	



Slaba

Osrednja

Minimalna podudarnost



Thinking Face With Question Mark Emoji

# Razlike u imunotestovima?!

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**Table 1.** Analytical specifications of the Abbott Architect and Mindray vitamin D assays.

Analytical specification	Abbott Architect 25-OH Vitamin D	Mindray 25-OH-Vitamin D total
Linearity (nmol/L)	8.5 - 389.8	10.0 - 374.4
Standardization	NIST SRM 2972	Not available
LOB (nmol/L)	4.0	≤ 7.5
LOD (nmol/L)	5.5	≤ 10.0
LOQ (nmol/L)	6.0	≤ 20.0
Interference	Interferent concentration	Interferent concentration
Hemoglobin	500 mg/dL	50 mg/dL
Bilirubin	30 mg/dL	20 mg/dL
Triglycerides	500 mg/dL	1500 mg/dL
Rheumatoid factor	800 IU/mL	400 IU/mL
Total protein	12 g/dL	10 g/dL
Biotin	30 ng/mL	Not declared
Cross-reactivity	(%)	(%)
25(OH)D <sub>3</sub>	98.6 - 101.1	98.0
25(OH)D <sub>2</sub>	80.5 - 82.4	96.3
1,25(OH) <sub>2</sub> D <sub>2</sub>	0	97.8
1,25(OH) <sub>2</sub> D <sub>3</sub>	0.1	97.9
24,25(OH) <sub>2</sub> D <sub>2</sub>	71.4 - 114.2	Not declared
24,25(OH) <sub>2</sub> D <sub>3</sub>	101.9 - 189.2	Not detectable
C3-epimer of 25OHD <sub>2</sub>	0.8	Not declared
C3-epimer of 25OHD <sub>3</sub>	1.3	48.0

LOB - limit of blank. LOD - limit of detection. LOQ - limit of quantification.

## Architect i2000SR

- Zečje monoklonsko At

## Mindray CL-1000i

- ???



# VDSP



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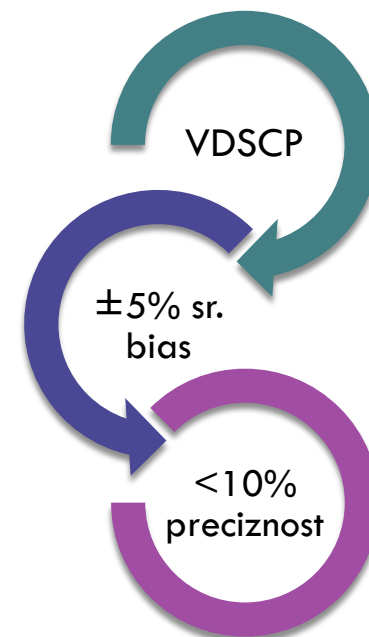
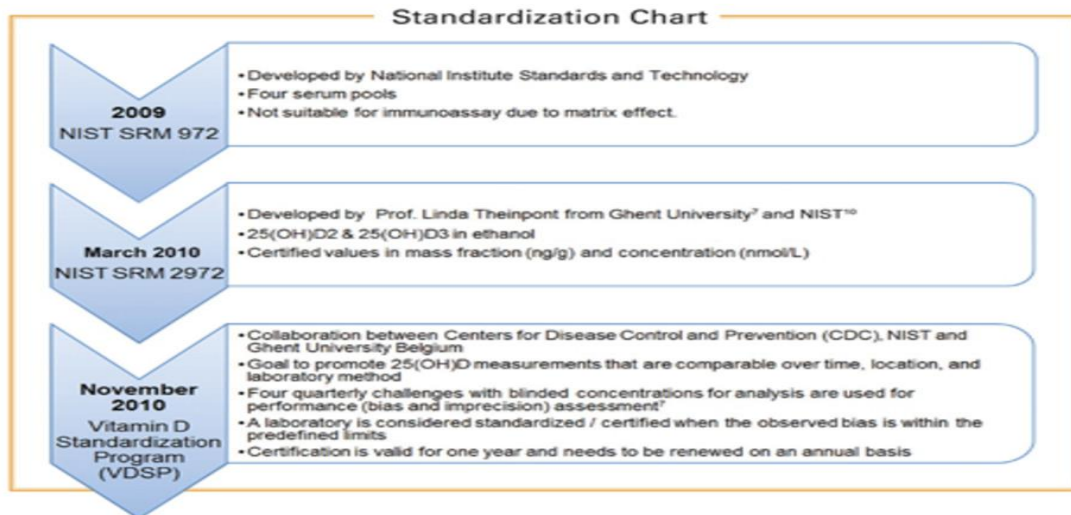
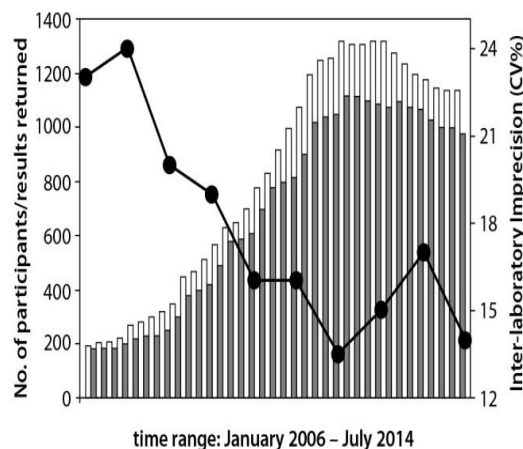


Figure 2



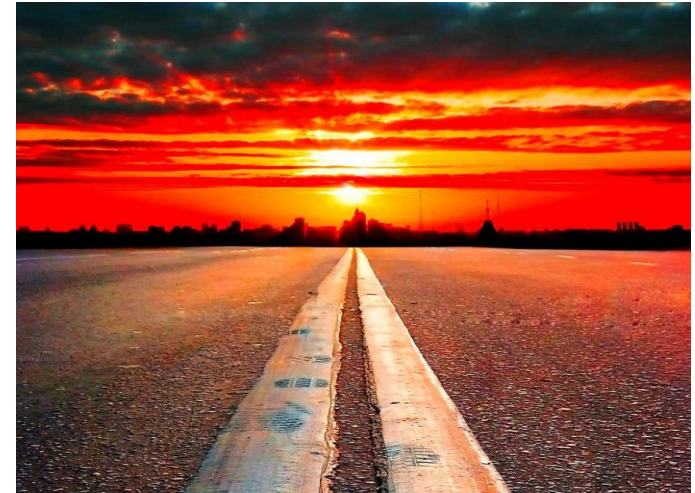
Relationship between the mean inter-laboratory imprecision (line and dotted plot) and the number of DEQAS (25-Hydroxyvitamin D measurements) registered laboratories (white bars). Gray bars indicates the numbers of returned results. With permission from DEQAS.

- Retrospektivna standardizacija 2 nacionalnih studija: The Third National Health and Nutrition Examination Survey (1988-1994) i The German Health Interview and Examination Survey for Children and Adolescents (2003-2006)

# Na horizontu...

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POCT (FDA, 2013.)





**You spend your time hiding in dark corners,  
Mr Cockroach, so of course, your blood test  
shows a lack of vitamin D: you need sunshine...**

**HVALA NA PAŽNJI!**