Varijabilnost imunokemijskih metoda i klinički značaj

Adriana Unić

Imunokemijske metode nisu savršene

- **Standardizacija** - rezultati dobiveni različitim metodama nisu usporedivi
- **Autoprotutijela** – epitopi na antigenu blokirani endogenim imunoglobulinima
- **Protutijela na reagens** – endogena nespecifična/multispecifična protutijela vežu se na reagens
- **Hook efekt** - izrazito visoke koncentracije analita dovode do zasićenja protutijela

Imunokemijske metode nisu usporedive

Clin Chem. 2004 Dec;50(12):2338-44. Epub 2004 Oct 7.

Performance characteristics of six third-generation assays for thyroid-stimulating hormone.

Rawlins ML[1], Roberts WL.

CONCLUSIONS: TSH methods do not provide comparable results for serum pools with TSH concentrations <0.2 mIU/L or for patient results across the analytic measurement range. Further investigation into the cause of these differences and additional harmonization efforts are required.

Am J Clin Pathol. 2007 Mar;127(3):436-40.

Performance characteristics of five automated CA 19-9 assays.

La'ulu SL[1], Roberts WL.

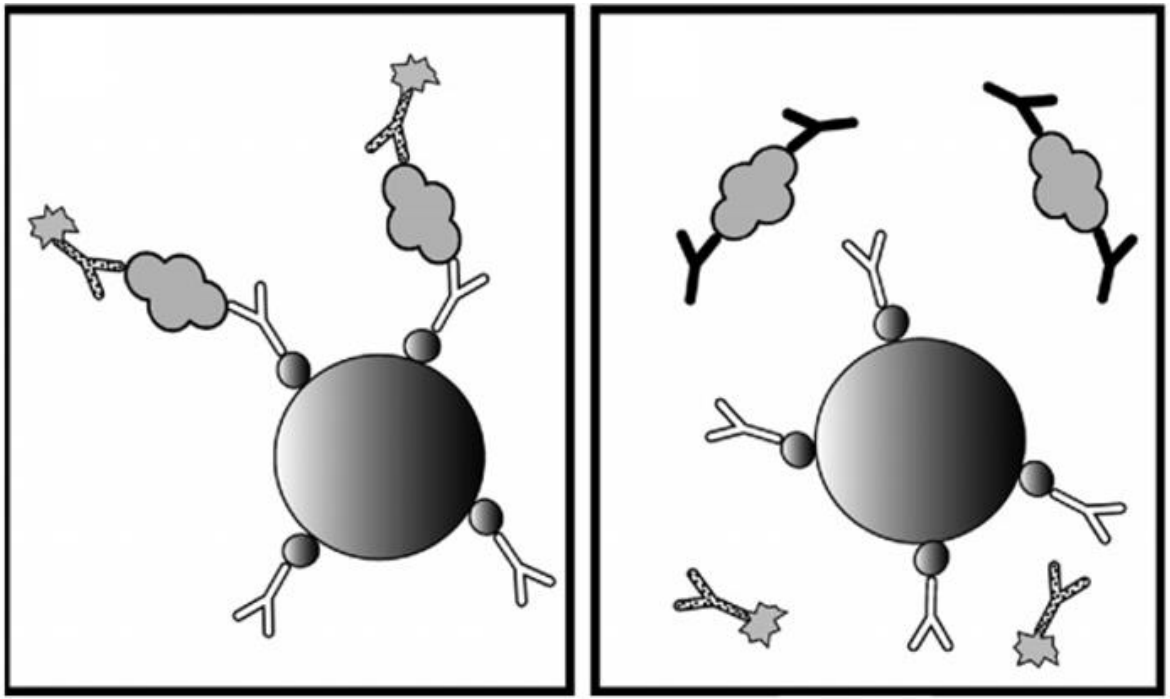
Serum concentrations of cancer antigen (CA) 19-9 can be useful in monitoring response to therapy in pancreatic cancer. The objective of this study was to evaluate 5 automated CA 19-9 assays: ARCHITECT 12000 (Abbott Diagnostics, Abbott Park, IL), ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY), UniCel Dxl 800 (Beckman Coulter, Fullerton, CA), IMMULITE 2000 (Diagnostic Products, Los Angeles, CA), and Elecsys E170 (Roche Diagnostics, Indianapolis, IN). All methods were evaluated for limit of detection, linearity, imprecision, method comparison and reference intervals. Limit of detection results were all below 2.0 kU/L and met the manufacturers' claims. Linearity had deviation from target values that ranged from 4.5% to 26.7%. All methods showed acceptable imprecision with total coefficients of variation less than 8%. Method comparison by Passing-Bablok analysis resulted in slopes ranging from 1.00 to 2.06 and correlation coefficients of 0.85 to 0.98. Between 97.6% and 99.2% of results from healthy volunteers were less than 35 kU/L. All methods show acceptable analytic performance.

Autoprotutijela

J Clin Endocrinol Metab. 1998 Apr;83(4):1121-7.

Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma.

Spencer CA[1], Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT.



J Immunol Methods. 2009 Aug 15; 347(1-2): 3–11.

Protutijela na reagens

Clin Chem. 1999 Jul;45(7):942-56.

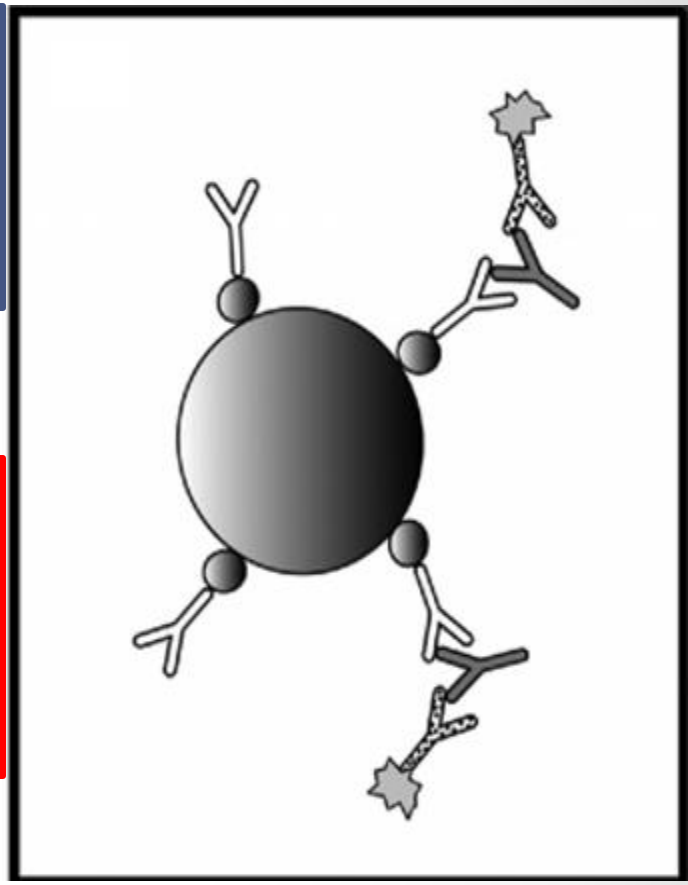
Human anti-animal antibody interferences in immunological assays.

Kricka LJ[1].

J Lab Clin Med. 1994 Mar;123(3):365-71.

Antibodies against biotinylated proteins are present in normal human serum.

Dale GL[1], Gaddy P, Pikul FJ.



J Immunol Methods. 2009 Aug 15; 347(1-2): 3–11.

Clin Chem Lab Med. 2007;45(3):416-8.

Efficacy of a new blocker against anti-ruthenium antibody interference in the Elecsys free triiodothyronine assay.

Sapin R, Agin A, Gasser F.

Hook efekt

J Neurooncol. 2006 Aug;79(1):41-3. Epub 2006 Apr 6.

Giant invasive pituitary prolactinoma with falsely low serum prolactin: the significance of 'hook effect'.

Fleseriu M[1], Lee M, Pineyro MM, Skugor M, Reddy SK, Siraj ES, Hamrahian AH.

Author information

Abstract

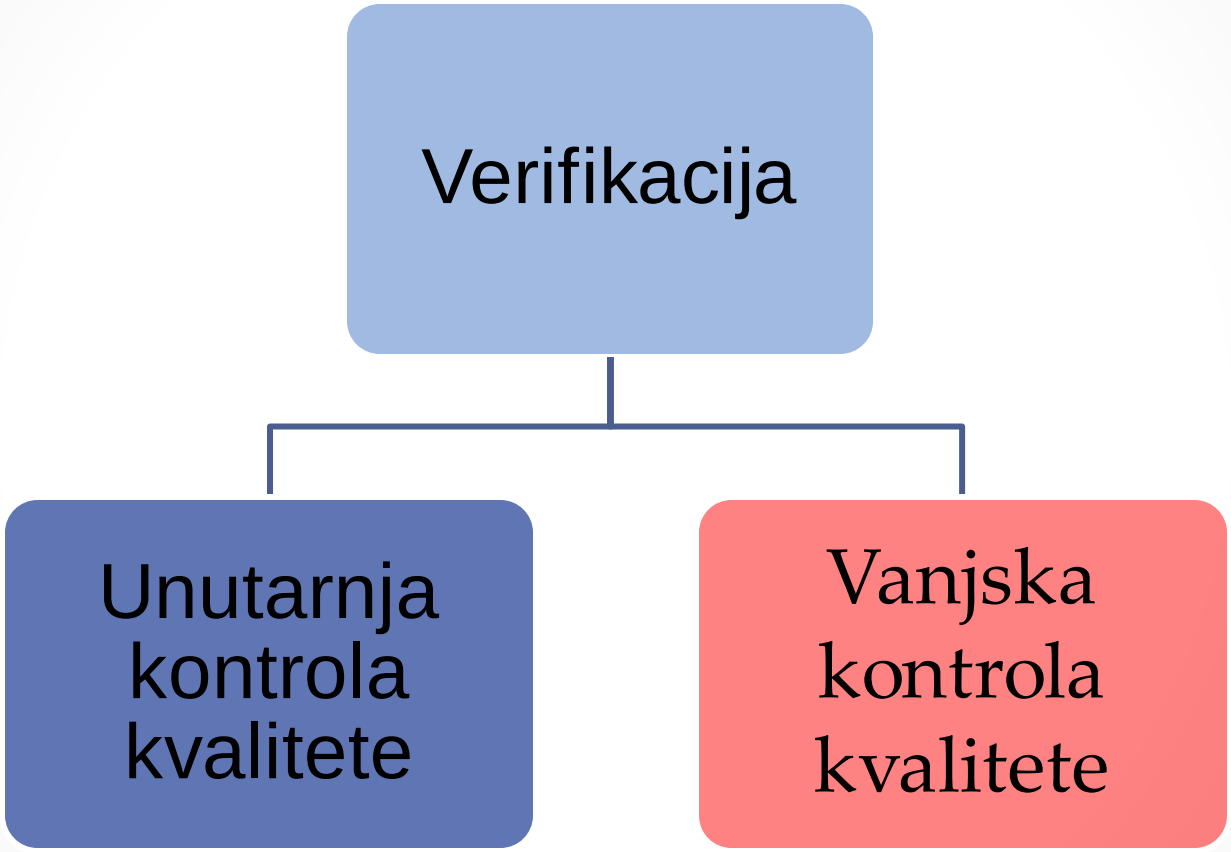
The authors report a case of a patient with giant, invasive skull base tumor extending to the parasellar area discovered incidentally during the work-up for decreased memory. The patient's neurological exam was otherwise unremarkable. Endocrine evaluation performed at a local hospital showed a moderate hyperprolactinemia 103 ng/ml (normal up to 20 ng/ml). Given the large size of the tumor, the elevated prolactin (PRL) was interpreted to be secondary to stalk effect and patient underwent debulking surgery through a transcranial approach. Immunostaining of the excised tumor tissue was strongly positive for prolactin. His prolactin was found to be 13,144 ng/ml in our lab after surgery confirming the diagnosis of invasive giant prolactinoma. The patient developed a complete right third, fourth and sixth nerve palsy postoperatively. He was started on Cabergoline with normalization of his prolactin level and more than 50% decrease in residual tumor size over 9 months periods. There has been no clinically significant improvement in his right eye ophthalmoplegia since surgery. This case highlights the importance of 'Hook Effect' resulting in falsely low prolactin level, which may have significant therapeutic implication.

J Urol. 2001 Jul;166(1):213.

High dose hook effect in serum total and free prostate specific antigen in a patient with metastatic prostate cancer.

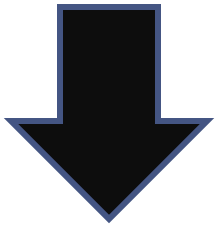
Furuya Y[1], Cho S, Ohta S, Sato N, Kotake T, Masai M.

Značajke izvedbe metode



Vanjska procjena analitičke kvalitete

Detekcija područja analitičkih problema



Stimulacija unaprijeđenja međulaboratorijske varijabilnosti

CROQALM

2013.-2016.

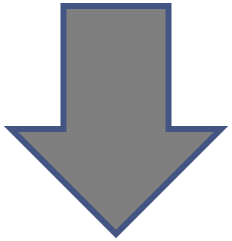
CROQALM 2013-2016

Analiza	Rezultati/ metode 2013	Rezultati/ metode 2016
CA 15-3	41/5	49/7
CA125	43/5	50/6
AFP	38/6	42/5
CEA	42/6	51/6
PSA	59/9	87/10
CA19-9	38/4	48/6
T4	41/9	47/8
TSH	60/10	90/9
T3	41/9	49/8
FT4	40/8	53/8
FT3	32/6	44/7
E2	24/4	33/4
fPSA	35/4	48/6
DHEA-S	12/3	24/3

Analiza	Rezultati/ metode 2013	Rezultati/ metode 2016
FSH	24/4	34/6
LH	24/3	33/4
Prog	21/4	32/5
Prolaktin	24/4	33/4
SHBG	12/3	21/4
Testo	20/4	33/6
Kortizol	19/5	31/5
HCG	33/5	45/6
Tg	9/2	12/3
PTH	25/4	32/5
Inzulin	9/3	16/3
C-peptid	7/3	13/3
Folati	13/3	21/3
B12	13/3	22/4

Problematika imunokemijskog modula

Mali broj ispitanika u jednoj „peer grupi”



Problem obrade podataka

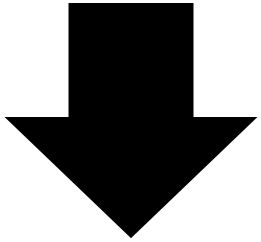
CROQALM 2013-2016

Analiza	Rezultati/ metode 2013	Rezultati/ metode 2016	N >/= 7
CA 15-3	41/5	49/7	3
CA125	43/5	50/6	3
AFP	38/6	42/5	3
CEA	42/6	51/6	3
PSA	59/9	87/10	4
CA19-9	38/4	48/6	3
T4	41/9	47/8	3
TSH	60/10	90/9	4
T3	41/9	49/8	3
FT4	40/8	53/8	3
FT3	32/6	44/7	3
E2	24/4	33/4	2
fPSA	35/4	48/6	3
DHEA-S	12/3	24/3	1

Analiza	Rezultati/ metode 2013	Rezultati/ metode 2016	N >/= 7
FSH	24/4	34/6	2
LH	24/3	33/4	2
Prog	21/4	32/5	1
Prolaktin	24/4	33/4	2
SHBG	12/3	21/4	1
Testo	20/4	33/6	1
Kortizol	19/5	31/5	2
HCG	33/5	45/6	3
Tg	9/2	12/3	1
PTH	25/4	32/5	1
Inzulin	9/3	16/3	1
C-peptid	7/3	13/3	1
Folati	13/3	21/3	2
B12	13/3	22/4	2

Problematika imunokemijskog modula

Unos rezultata u pogrešnim jedinicama



Pogrešna interpretacija obrade rezultata

Problematika imunokemijskog modula

Assay	Factor	Primary standard
Abbott Architect	21	3rd IS 84/500
Abbott Axym	24	3rd IS 84/500
Adaltis ElAgen	21.2	3rd IS 84/500
Beckman Coulter UniCel	21.2	
Beckman Coulter Immunotech	30.3	3rd IS 84/500
bioMerieux Vidas	22	
Diasorin Liaison	21.2	3rd IS 84/500
DRG Diagnostics ELISA	21.2	3rd IS 84/500
DRG Prolactin IRMA		1st IRP 75/504
Perkin Elmer AutoDefia	36	3rd IS 84/500
Roche Elecsys&Modular E&cobas	21.2	3rd IS 84/500
Siemens Advia Centaur	21.2	3rd IS 84/500
Siemens Immulite	21.2	3rd IS 84/500
TOSOH AIA	27	

PRETRAGA: **PROLAKTIN (PRL), IU/L**

	Statistička obrada					
Metoda	N	- N	median	MIN	MAX	X
Bez obzira na metodu	24	3	1170	1	2133	980,0
Abbott Architect-CMIA	7	0	1028	45	2133	981,1
Biomerieux Vidas/mini Vidas-ELFA	1	0	30	30	30	30,0
Beckman Coulter-CLIA	3	0	575	30	682	429,0
Roche Elecsys/Modular/Cobas-ECLIA	13	3	1237	1	1397	1.239,4

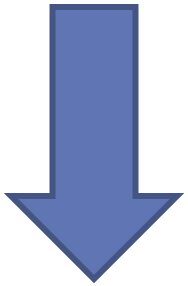
660 IU/L

945 IU/L

636 IU/L

Zaključci obrade imunokemijskog modula

Metode nisu međusobno usporedive



Utjecaj na interpretaciju rezultata

CA 15-3

CA15-3	2013/1	2014/1	2014/3	2015/1	2015/3	2016/1
Rezultati/ metode	41/5	48/6	50/6	52/6	51/6	49/7
Median svih metoda	65.95	18.5	32.5	76.0	41.7	24.7
Median BC– CLIA (broj rezultata)	36.3 (5)	13.2 (6)	16.1 (5)	30.6 (7)	18.0 (7)	16.1 (9)

Am J Clin Pathol 2006;125:752-757

Analytic Concordance With the ADVIA Centaur CA 15-3 Method*

	ADVIA Centaur CA 15-3			Overall Concordance With ADVIA Centaur (%)
	≥32.4 kU/L	<32.4 kU/L	Total	
Access 2				89.0
≥31.3 kU/L	63	0	63	
<31.3 kU/L	11	26	37	
Total	74	26	100	
ARCHITECT i2000				95.0
≥31.3 kU/L	69	0	69	
<31.3 kU/L	5	26	31	
Total	74	26	100	
AxSYM				94.0
≥31.3 kU/L	71	3	74	
<31.3 kU/L	3	23	26	
Total	74	26	100	
Elecsys 2010				89.0
≥25 kU/L	74	11	85	
<25 kU/L	0	15	15	
Total	74	26	100	
IMMULITE 2000				96.0
≥38.0 kU/L	74	7	81	
<38.0 kU/L	0	19	19	
Total	74	26	100	
VITROS ECi				93.0
≥35 kU/L	74	7	81	
<35 kU/L	0	19	19	
Total	74	26	100	

CA 19-9

CA 19-9	2013/1	2014/1	2014/3	2015/1	2015/3	2016/1
Rezultati/ metode	38/4	47/6	49/6	51/6	50/6	48/6
Median svih metoda	141	20.8	56.1	134.2	58.0	31.0
Median - Abbott Archictect	482.9 (11)	73.3 (12)	188.0 (12)	467.3 (12)	197.4 (12)	166.6 (11)

French Agency for the Safety of Health Products

„...a complaint was registered upon the request of the country regulatory authority afssaps stating that **a review of the national quality control data indicated a systematic difference in results was observed between results generated using the Architect assay compared to results reported using other techniques on the market for this assay.** An example of control data was provided: **the mean result of the architect CA 19-9 method was 59 and the mean of the results of the other methods/techniques was 23 with a range from 20 to 33 (units of measure not provided but assumed to be u/ml)**. Afssaps questioned if the decisional cut-off value of 37 is correct for the architect ca assay. It was also mentioned that the result differences between architect and other methods/techniques is causing a problem for patients that have to have their ca assay run in different laboratories using various techniques. **It was stated that the architect value could be pathological and the other techniques could show a normal value.** Afssaps is requesting an explanation regarding these value differences that do not satisfy the data included in the Architect CA package insert/labeling. **No patient specific data was provided.** No adverse impact to patient management was reported due to this issue..."

Odgovor proizvođača

„...The Architect CA19-9xr assay may not agree with other methods when testing patient samples and proficiency samples. **In general, the Architect CA19-9xr assay tends to exhibit higher concentration values for cancer patients than by other CA19-9 methods.** However, **our data has also shown that the architect CA19-9xr assay may provide lower values than other CA19-9 methods for samples toward the low concentration end of the range.** Because of these differences between platforms, patients must be re-baselined when changing between assay methods during follow-up care. **The differences between architect CA 19-9xr and other methods are due to several key design elements regarding assay standardization and reagent configuration that have resulted in greater capture and detection of CA 19-9 from human serum, reduced interference from human anti-idiotype antibodies, and lower background signal.**

Odgovor proizvođača

Some patients may have CA19-9 having a greater number of reactive antigenic determinants than others, and would be expected to produce higher concentration values on the architect platform than by other methods that use whole molecule antibody conjugates. **The same is true for proficiency sample and external qc controls when these controls are made using pooled patient specimens because some of the units in the pool may contain CA19-9 with a high number of repeating reactive antigenic determinants. Microparticle reagent - the architect CA19-9xr microparticle reagent formulation has been optimized for pH and antibody coating concentration to maximize capture of CA 19-9 onto the solid phase. The use of an acidic pH for the reaction mixture together with an increased antibody coating concentration allows more ca19-9 binding to the microparticle. Proficiency panel & external qc control considerations: the nature of the antigen used in the proficiency panel or externally supplied qc controls may contribute to differences in their concentration values across assay methods. .. The literature review of ca 19-9 assay differences provided additional evidence that this assay is performing as intended. No additional issues were identified during this investigation, and no further investigation is required. This is the final report."**

Am J Clin Pathol 2007;127:436-440

- Performance Characteristics of Five Automated CA 19-9 Assays

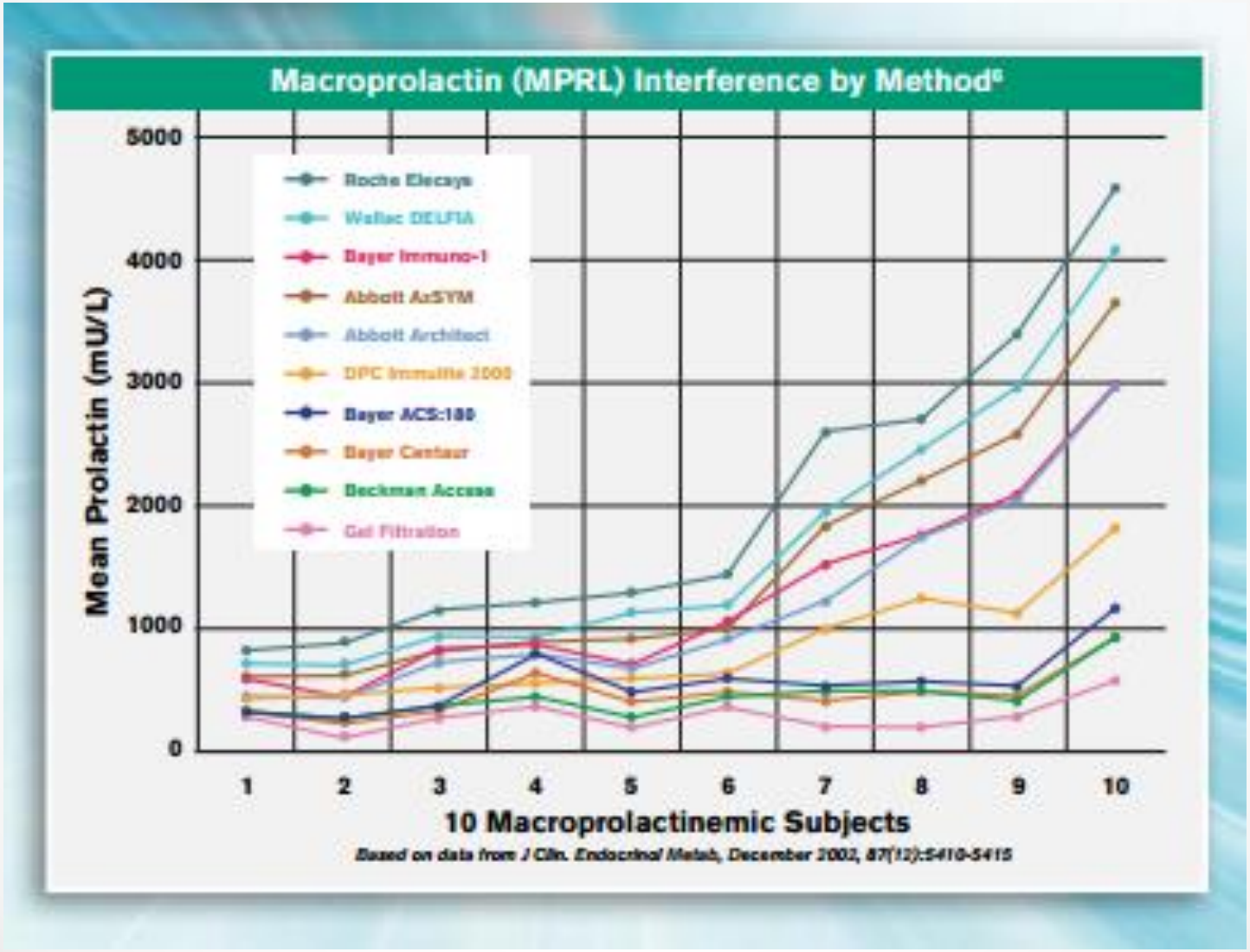
Summary of Reference Interval Data

Method*	Range of Results (kU/L)	Median (kU/L)	97.5th Percentile (kU/L)	Percentage <35 kU/L
ADVIA Centaur	4.3-56.8	9.7	4.7-37.1	97.6
ARCHITECT *i*2000	2.0-60.3	4.4	2.0-26.4	98.4
IMMULITE 2000	2.5-38.0	3.3	2.5-17.0	99.2
Elecsys E170	0.6-46.3	6.9	0.6-31.9	99.2
UniCel Dxl 800	1.0-51.0	7.0	1.0-33.2	99.2

PROLAKTIN

Prolaktin (mIU/L)	2013/1	2014/1	2014/3	2015/1	2015/3	2016/1
Rezultati/ metode	24/4	29/5	30/7	29/5	32/5	33/4
Median svih metoda	1170	119	667	1353	797	179
Beckman Coulter - CLIA	575 (3)	88 (4)	395 (4)	680 (5)	432 (5)	122 (6)

PROLAKTIN


Macroprolactin (MPRL) Interference by Method[6]
Mean Prolactin (mU/L)
5000
4000
3000
2000
1000
0
Roche Elecsys
Wallac DELFIA
Bayer Immuno-1
Abbott AxSYM
Abbott Architect
DPC Immulite 2000
Bayer ACS:180
Bayer Centaur
Beckman Access
Gel Filtration
1
2
3
4
5
6
7
8
9
10
10 Macroprolactinemic Subjects
Based on data from J Clin. Endocrinol Metab, December 2002, 87(12):5410-5415

CEA

PRETRAGA: **CEA, µg/L**

Metoda	Statistièka obrada N	- N	median	MIN	MAX	X
Bez obzira na metodu	42	2	39,25	8,1	59,5	35,12
Abbott Architect-CMIA	11	1	55,65	51,7	59,5	55,95
Siemens Bayer Advia Centaur-CLIA	4	0	8,6	8,4	9,6	8,80
Biomerieux Vidas/mini Vidas-ELFA	2	0	8,35	8,1	8,6	8,35
Beckman Coulter-CLIA	4	0	9,4	8,6	9,9	9,32
Roche Elecsys/Modular/Cobas-ECLIA	20	1	39,3	33,8	43,7	39,23
Siemens Immulite CLIA	1	0	10,5	10,5	10,5	10,50

2013/1

PRETRAGA: **CEA, µg/L**

Metoda	Statistička obrada N	- N	median	MIN	MAX	X
Bez obzira na metodu	50	0	6,05	4,5	8,4	6,0
Abbott Architect-CMIA	12	0	6,8	6,1	7,6	6,8
Siemens Bayer Advia Centaur-CLIA	3	0	6,4	6,4	7,4	6,7
Biomerieux Vidas/mini Vidas-ELFA	2	0	5,2	5,2	5,2	5,2
Beckman Coulter-CLIA	5	0	6,4	6,2	6,6	6,4
Roche Elecsys/Modular/Cobas-ECLIA	23	0	5,1	4,5	6	5,2
Siemens Immulite CLIA	5	0	7,3	6,3	8,4	7,3

2014/1

PRETRAGA: **CEA, µg/L**

Metoda	Statistička obrada N	- N	median	MIN	MAX	X
Bez obzira na metodu	52	15	18,4	3,6	28,3	20,1
Abbott Architect-CMIA	12	2	23,8	21,1	26,2	23,7
Siemens Bayer Advia Centaur-CLIA	5	5		3,9	4,3	
Biomerieux Vidas/mini Vidas-ELFA	1	1		3,6	3,6	
Beckman Coulter-CLIA	5	5		4,2	4,4	
Roche Elecsys/Modular/Cobas-ECLIA	25	1	17,75	16,2	20,4	17,8
Siemens Immulite CLIA	4	1	28	4,3	28,3	26,6

2014/3

CEA

PRETRAGA: **CEA, µg/L**

Metoda	N	- N	Statistička obrada median	MIN	MAX	X
Bez obzira na metodu	53	1	40,65	14,5	71,1	40,2
Abbott Architect-CMIA	12	0	55,5	52,9	61,5	56,0
Siemens Bayer Advia Centaur-CLIA	5	0	15,7	14,5	18,3	15,8
Biomerieux Vidas/mini Vidas-ELFA	1	0	16	16	16	16,0
Beckman Coulter-CLIA	6	0	15,85	14,8	16,4	15,7
Roche Elecsys/Modular/Cobas-ECLIA	25	1	40,35	37,3	46,2	40,5
Siemens Immulite CLIA	4	0	63,25	59,2	71,1	64,2

2015/1

PRETRAGA: **CEA, µg/L**

Metoda	N	- N	Statistička obrada median	MIN	MAX	X
Bez obzira na metodu	52	2	18,3	4,8	32	17,7
Abbott Architect-CMIA	11	1	25,1	17,8	26,8	25,1
Siemens Bayer Advia Centaur-CLIA	5	0	7,6	4,8	8,4	7,2
Biomerieux Vidas/mini Vidas-ELFA	1	0	7,5	7,5	7,5	7,5
Beckman Coulter-CLIA	7	0	7,6	7,2	8,3	7,7
Roche Elecsys/Modular/Cobas-ECLIA	24	1	18,3	14,9	20,5	18,3
Siemens Immulite CLIA	4	0	29,9	24,3	32	29,0

2015/3

PRETRAGA: **CEA, µg/L**

Metoda	N	- N	Statistička obrada median	MIN	MAX	X
Bez obzira na metodu	51	0	3,3	2,4	4	3,3
Abbott Architect-CMIA	11	0	3,3	3	3,8	3,4
Siemens Bayer Advia Centaur-CLIA	3	0	2,9	2,4	3,3	2,9
Biomerieux Vidas/mini Vidas-ELFA	2	0	2,85	2,6	3,1	2,9
Beckman Coulter-CLIA	9	1	3,05	2,9	3,6	3,0
Roche Elecsys/Modular/Cobas-ECLIA	23	0	3,4	3	3,8	3,4
Siemens Immulite CLIA	3	0	3,7	3,6	4	3,8

2016/1

CEA

	ACS:Centaur		Architect		Elecsys 2010		Immulite 2000		Vitros Eci	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
CEA	2.4	4.4	3.3	3.5	3.3	5.1	2.9	3.1	-	-
µg/l	16.4	2.7	19.6	2.7	21.1	3	19.8	2.8	-	-
	36.5	2.1	42.1	2.5	45.2	2.5	53.7	2.8	-	-

Ned Tijdschr Klin Chem 2000; 25: 170-177

I na kraju...

- **Imunokemijske metode nisu savršene**

- **Važno je poznavati i detektirati njihova ograničenja**