Pièges cliniques et biologiques en endocrinologie

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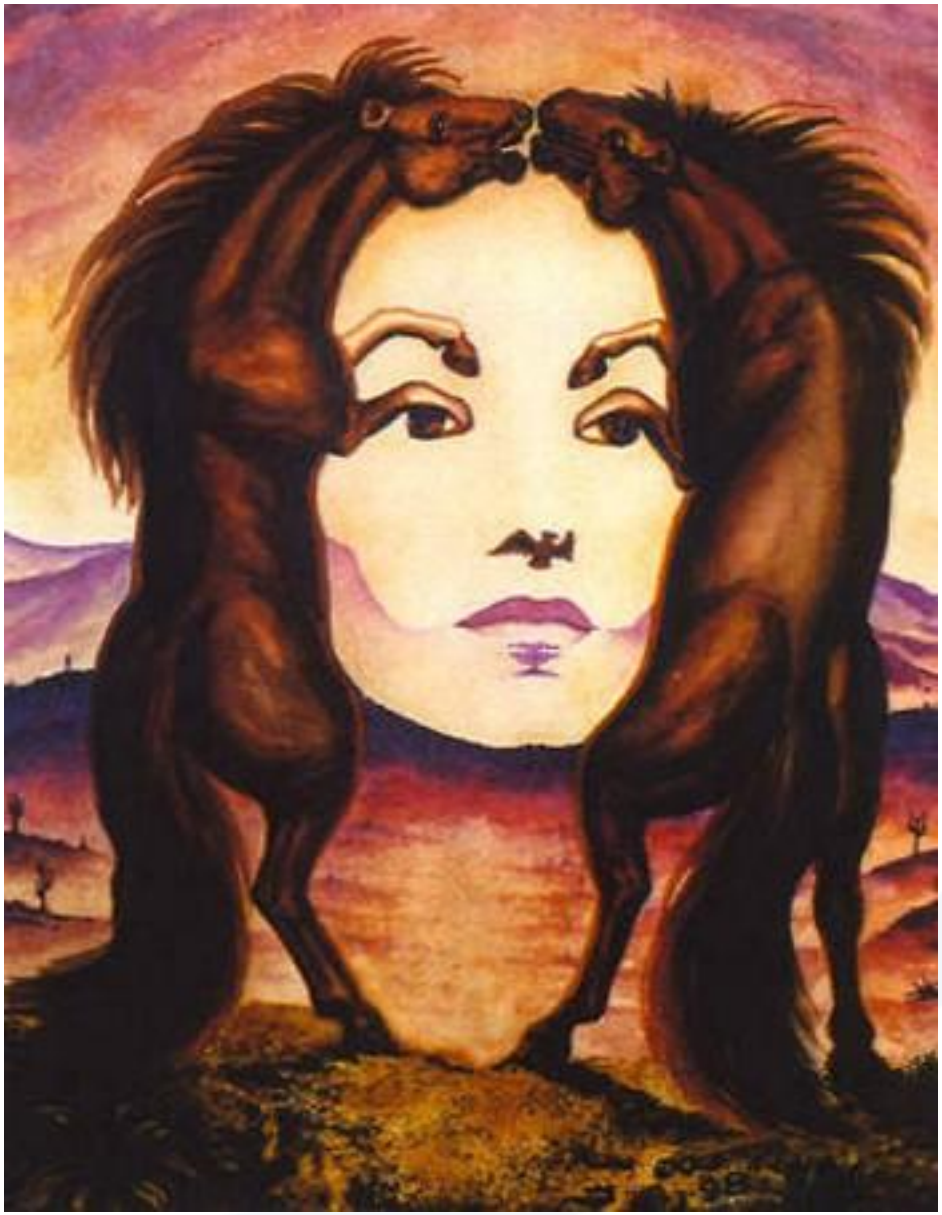
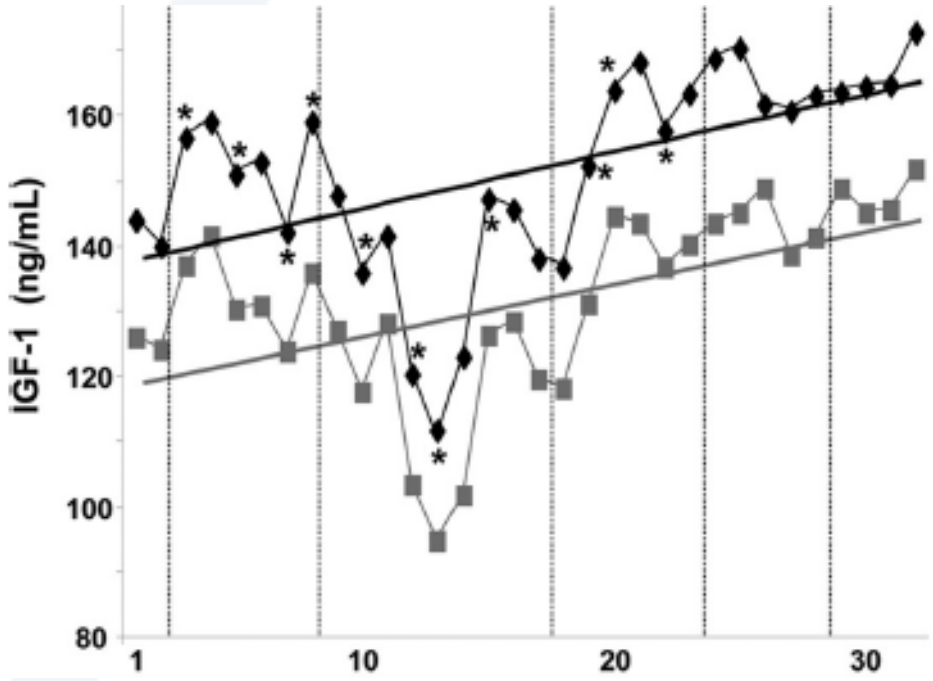
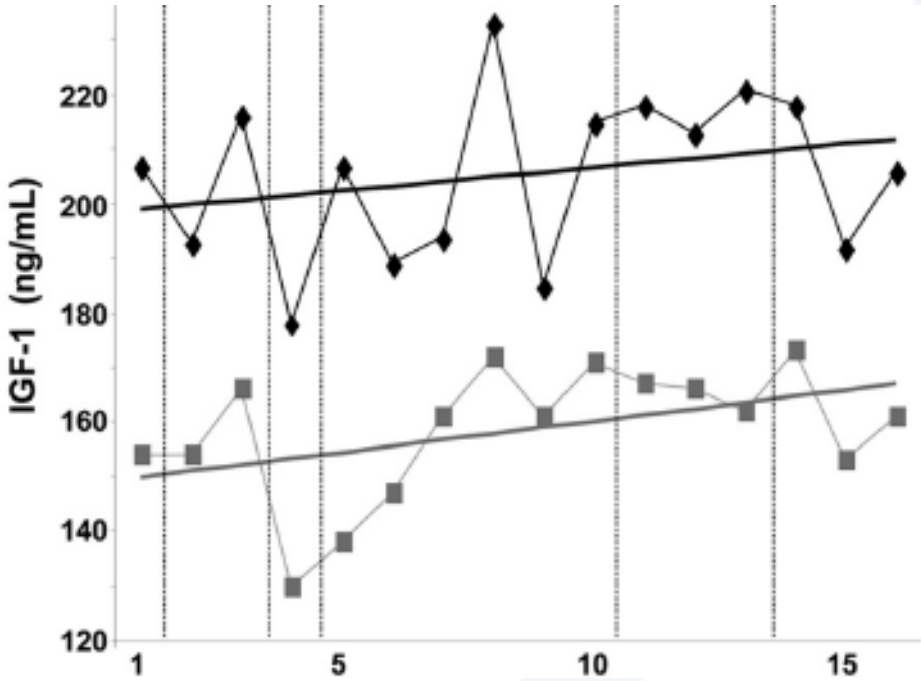



IGF-1 (ng/mL)
160
140
120
100
80
1
10
20
30


IGF-1 (ng/mL)
220
200
180
160
140
120
1
5
10
15

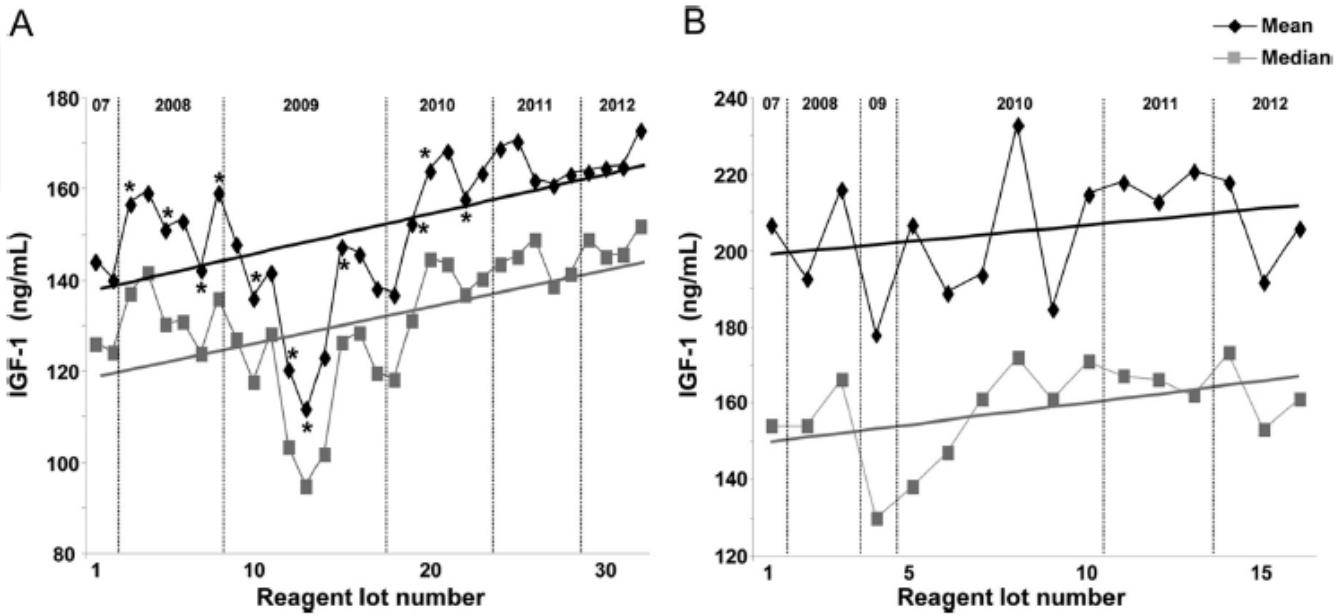


Table 1. Comparison between the first and last IGF-1 reagent lot used at Mayo Clinic.[a]

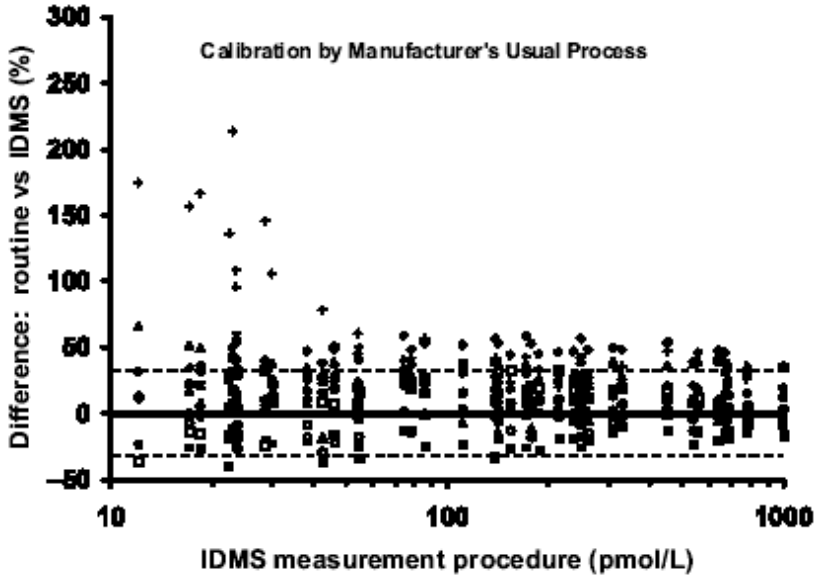
	Lot 1[b]	Lot 32	*P* for difference
IGF-1, ng/mL	126 (90–178)	156 (102–219)	<0.0001
Result distribution			
Above reference interval	212 (9.2)	621 (17.2)	<0.0001
Within reference interval	1572 (68.6)	2364 (65.4)	
Below reference interval	508 (22.2)	628 (17.4)	
Patient age, years			
21–25	120 (5.2)	220 (6.1)	0.0854
26–30	133 (5.8)	241 (6.7)	
31–35	164 (7.2)	299 (8.3)	
36–40	199 (8.7)	319 (8.8)	
41–45	256 (11.2)	360 (10.0)	
46–50	297 (12.9)	411 (11.4)	
51–55	255 (11.1)	426 (11.8)	
56–60	268 (11.7)	410 (11.3)	
61–65	203 (8.9)	329 (9.1)	
66–70	136 (5.9)	261 (7.2)	
71–75	128 (5.6)	169 (4.7)	
76–80	73 (3.2)	106 (2.9)	
>80	60 (2.6)	62 (1.7)	

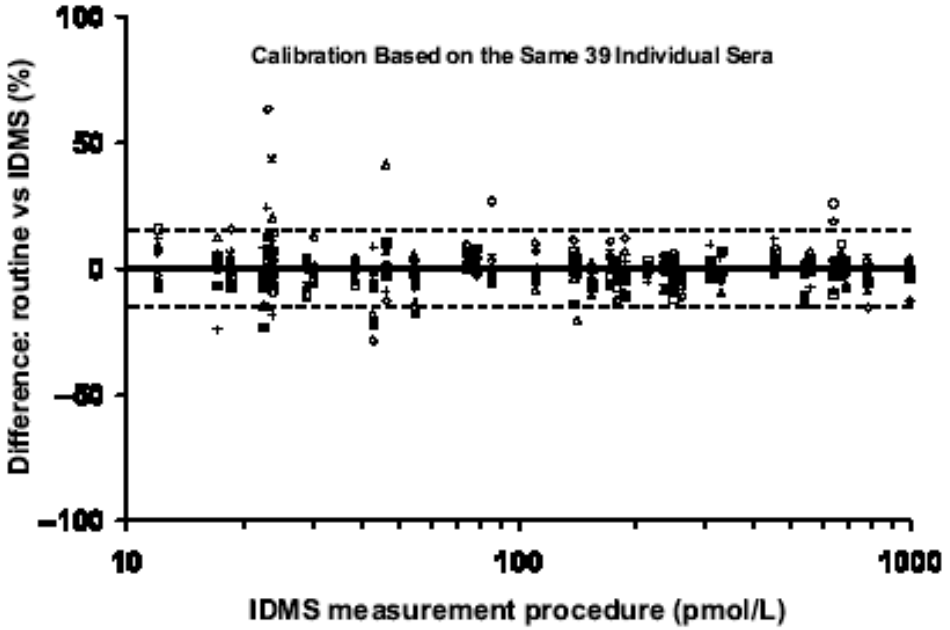
[a] Data are median (interquartile range) or n (%).
[b] Corresponding manufacturer reagent lot numbers for lots 1 and 32 are 401 and 481.

Algeciras-Schimnich et al ; 2014

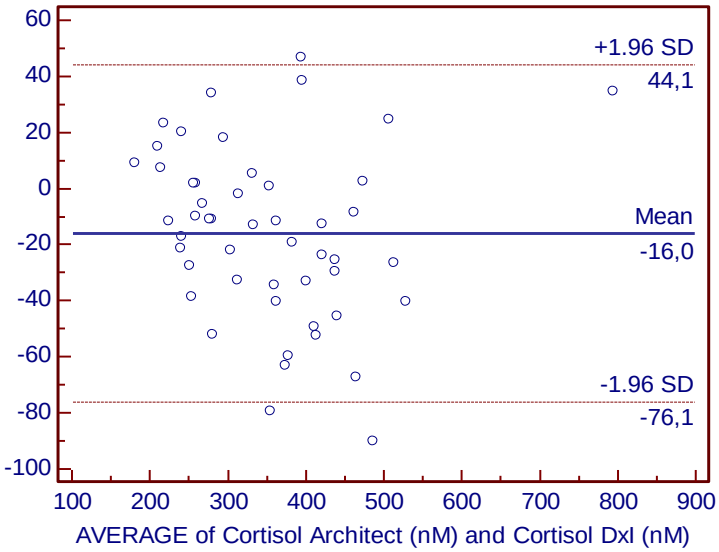
Toward Standardization of Insulin Immunoassays

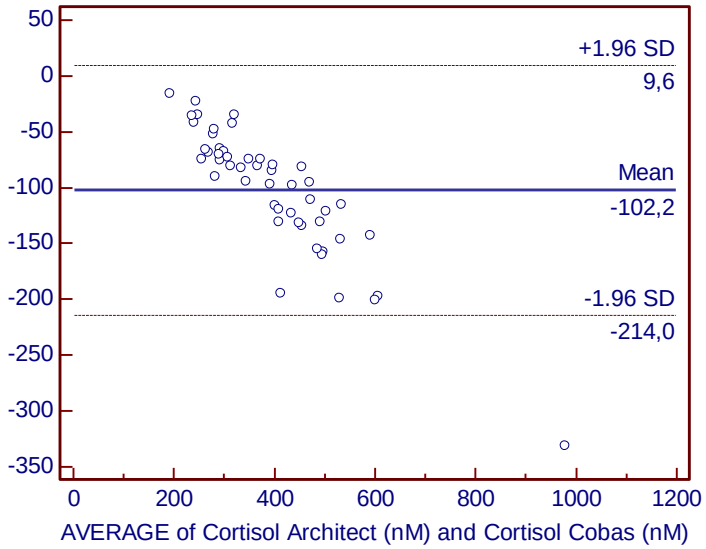
W. Greg Miller,[1*] Linda M. Thienpont,[2] Katleen Van Uytfanghe,[2] Penelope M. Clark,[3] Patrik Lindstedt,[4] Göran Nilsson,[5] and Michael W. Steffes,[6] for the Insulin Standardization Work Group

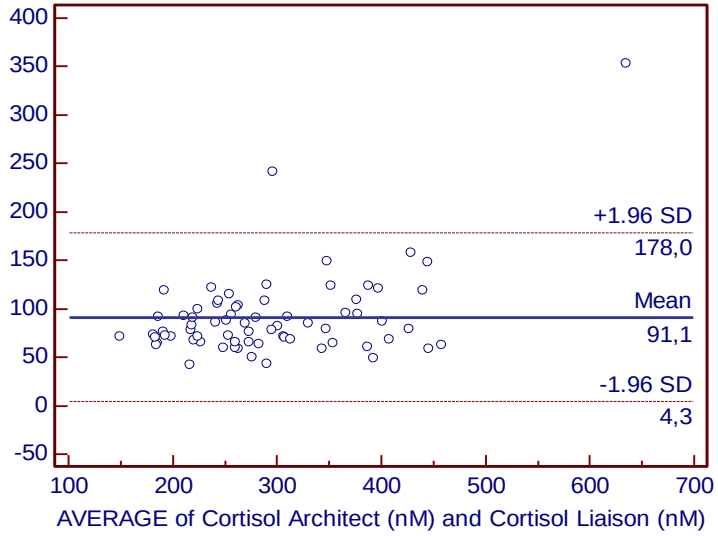


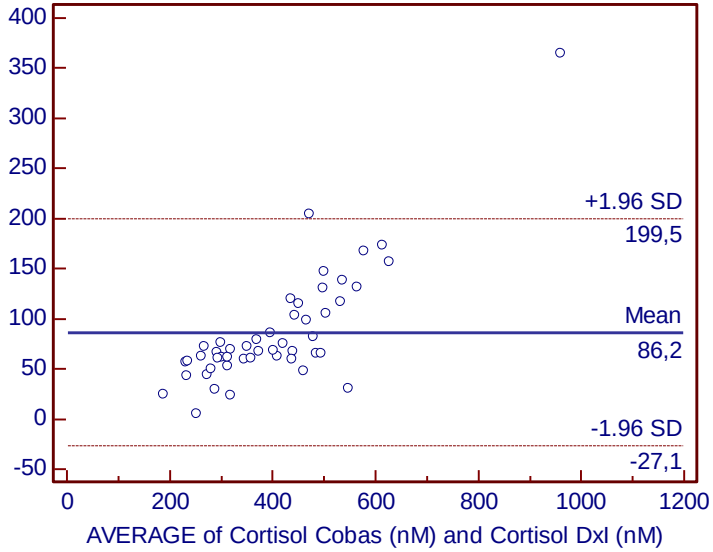


Serum / Plasma cortisol testing









Factors of Variability

Analytical Reliability

Uncontrollable Factors

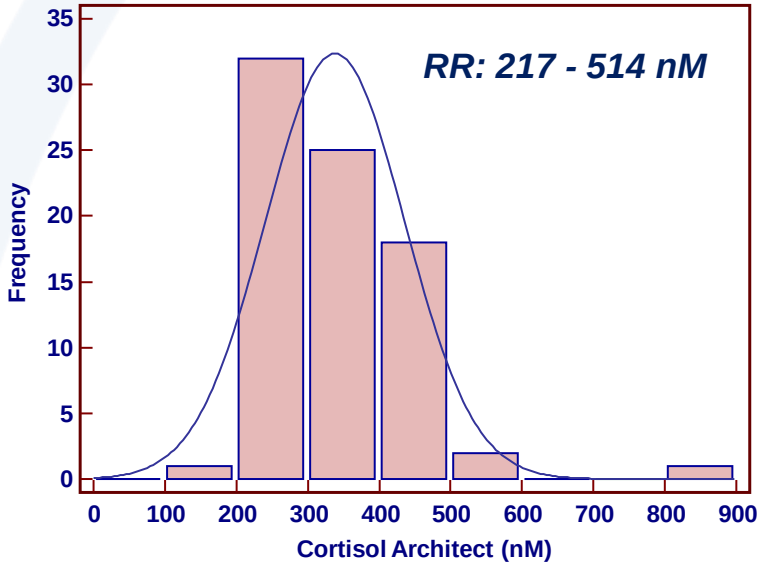
- → Age
- → Gender
- → Menopausal status
- → Disease

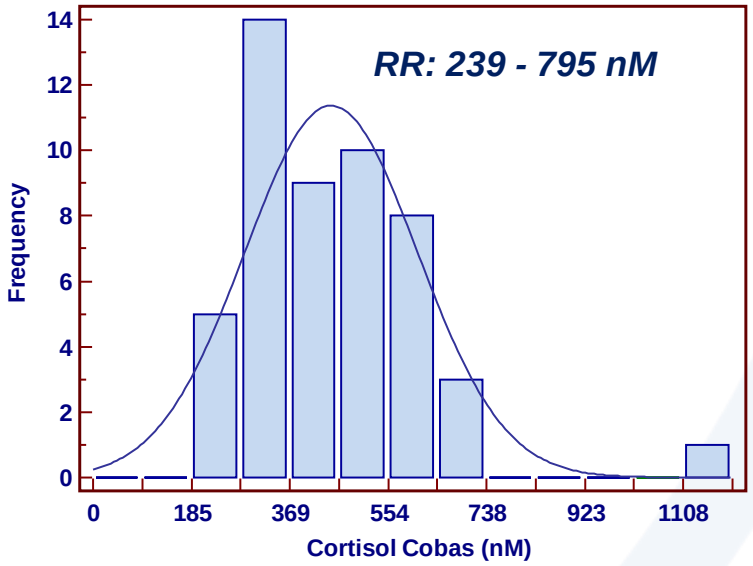
Controllable Factors of Variability

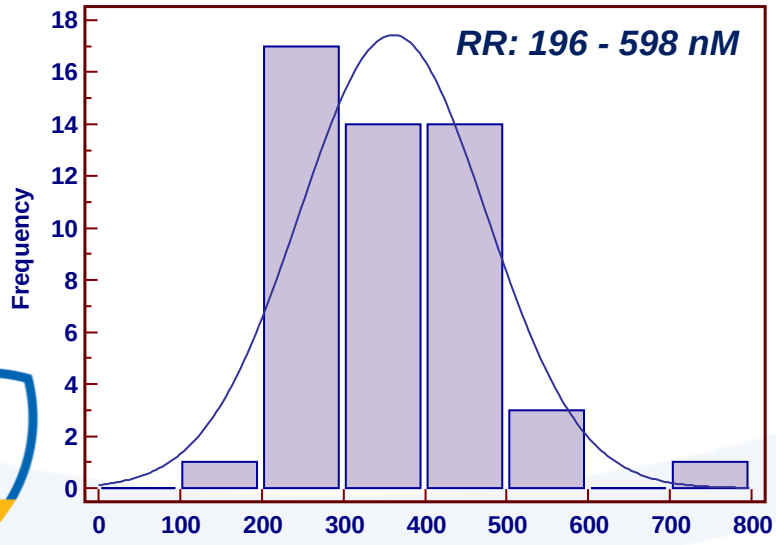
- →Circadian
- →Menstrual
- →Nutritional

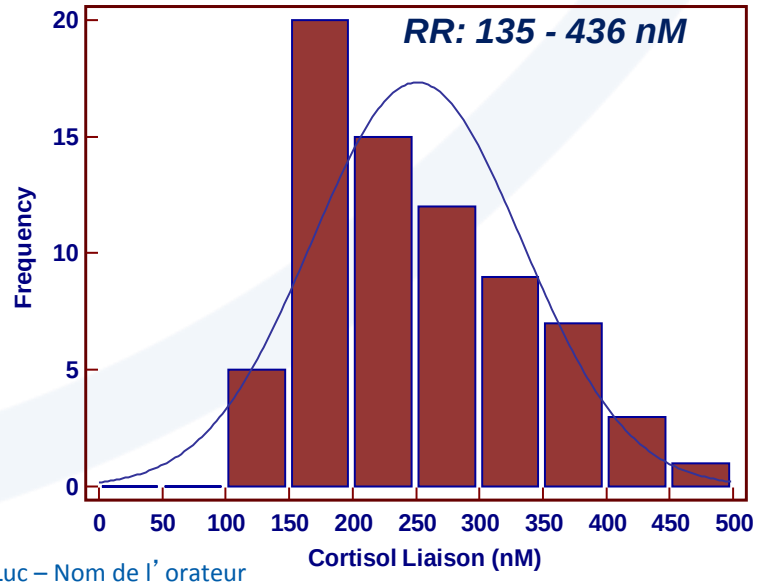
Accounting the effects by appropriate reference ranges, quality control policies, standardization…

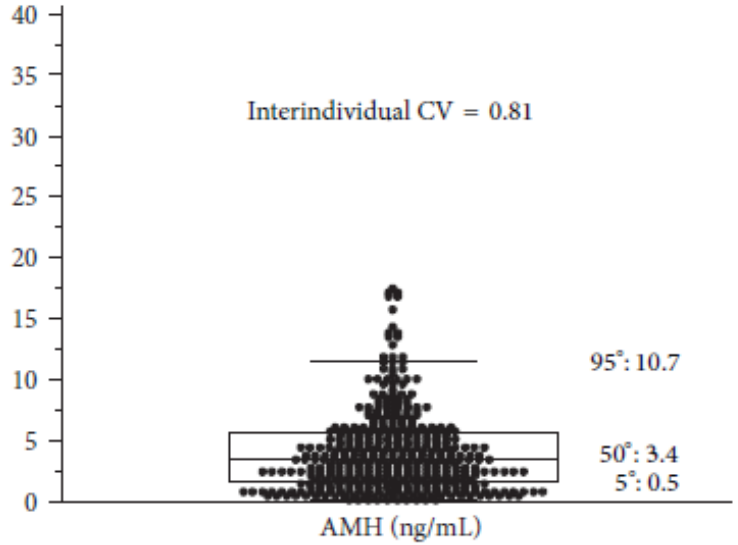
Serum / Plasma cortisol testing

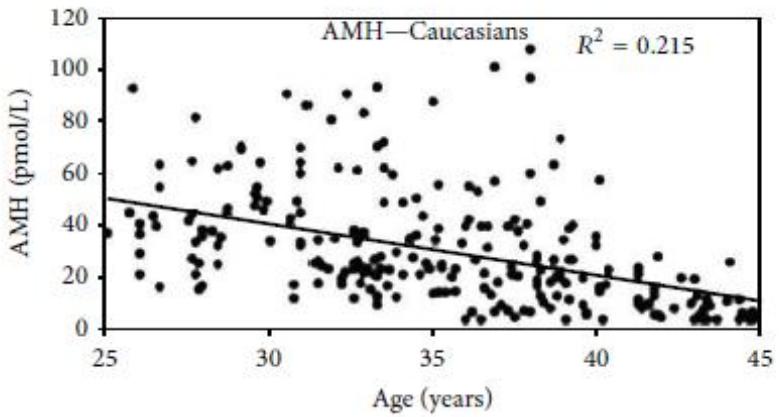




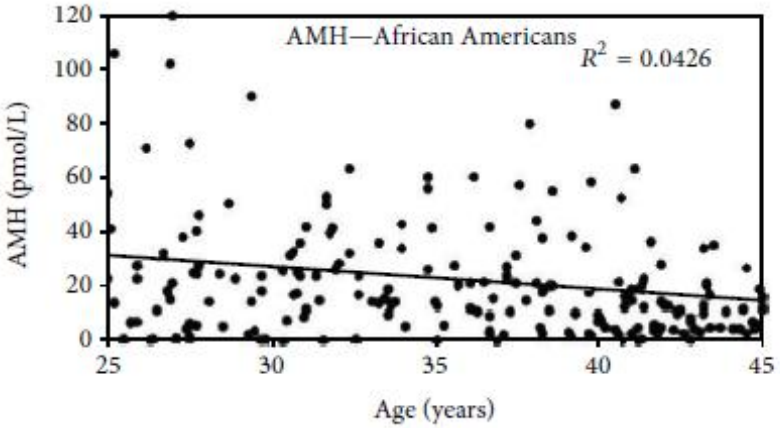








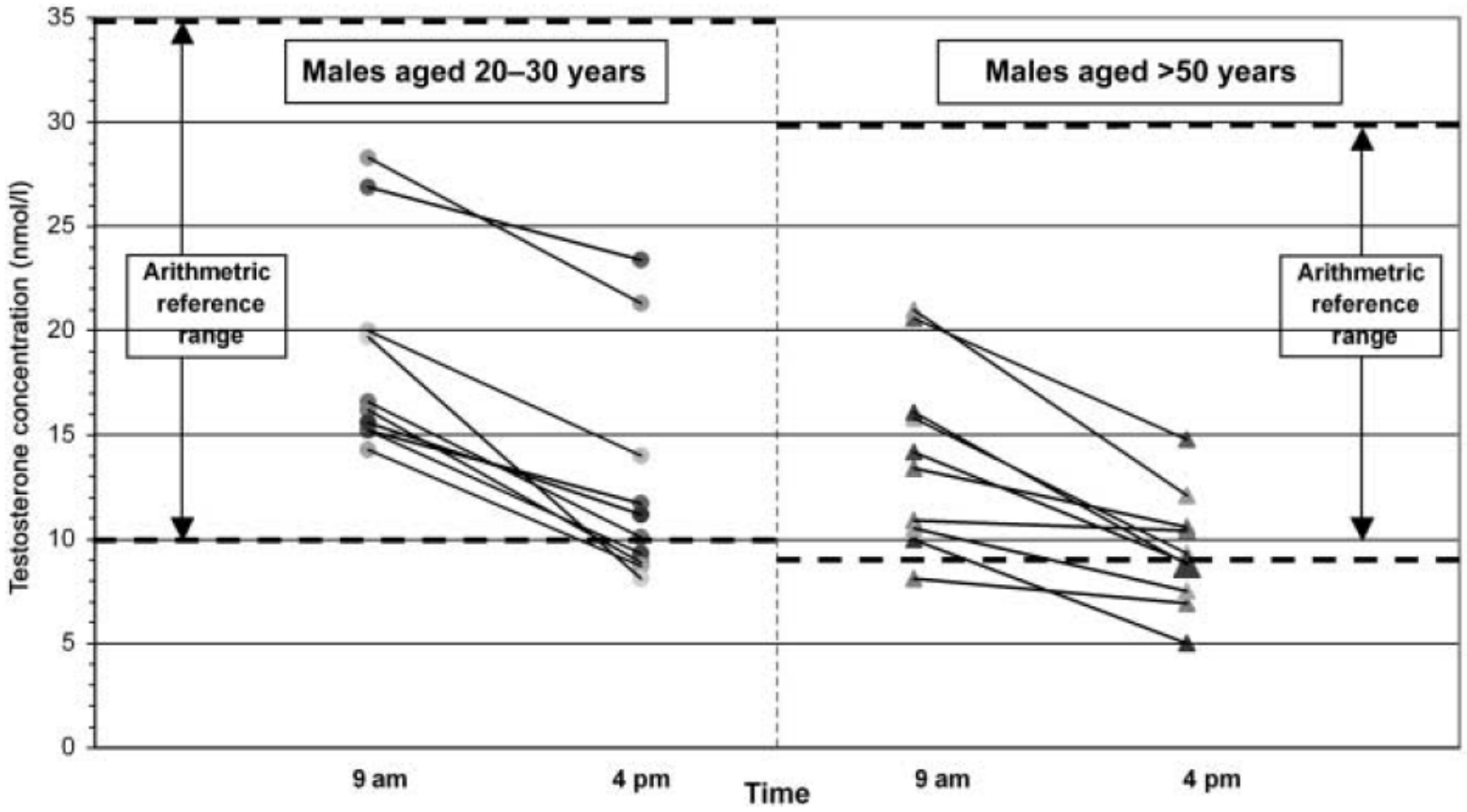
Femmes	Hommes
0-16 ans: 0.1-7.1 ng/mL 17-29 ans: 0.85-14.24 ng/mL 30-39 ans: 0.51-7.27 ng/mL 40-49 ans: 0.1-6.21 ng/mL Au delà de 50 ans: 0.1-0.82 ng/mL	0-13 jours: 15.50-48.10 ng/mL 14 jours-11 mois: 39.10-91.10 ng/mL 12 mois-6 ans: 48.00-83.20 ng/mL 7-8 ans: 33.80-60.20 ng/mL 9-12 ans: 6.1-60.7 ng/mL 13-16 ans: 2.3-33.1 ng/mL 17 ans et plus: 1.50-18.35 ng/mL



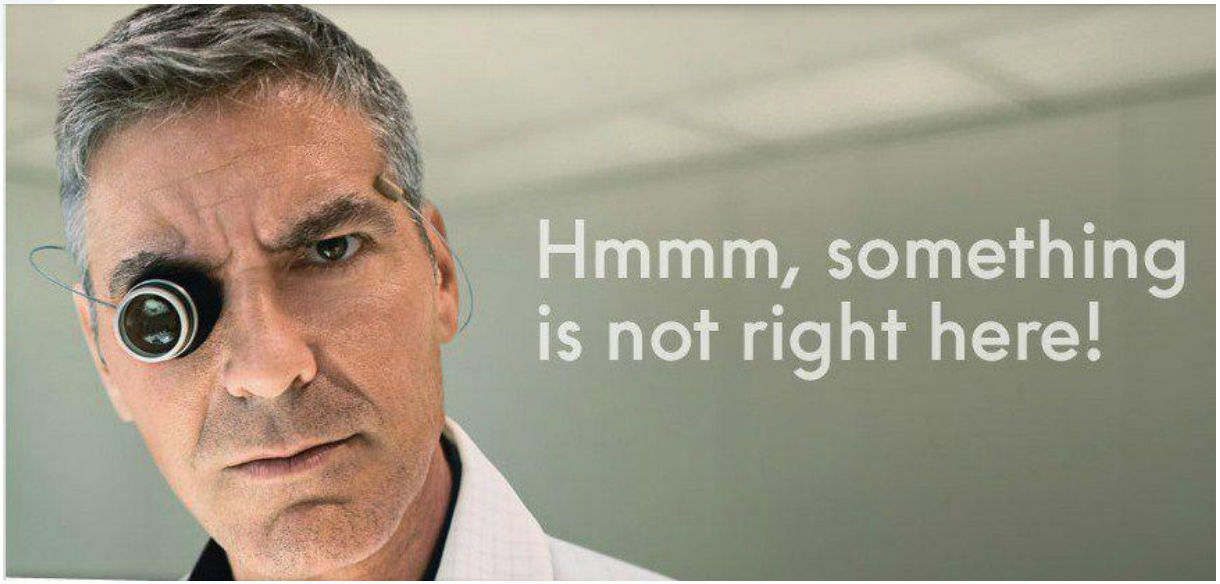
La Marca et al., 2014

Testosterone assay

Pre-analytic



Wheeler et al., Clinical Endocrinology 2008

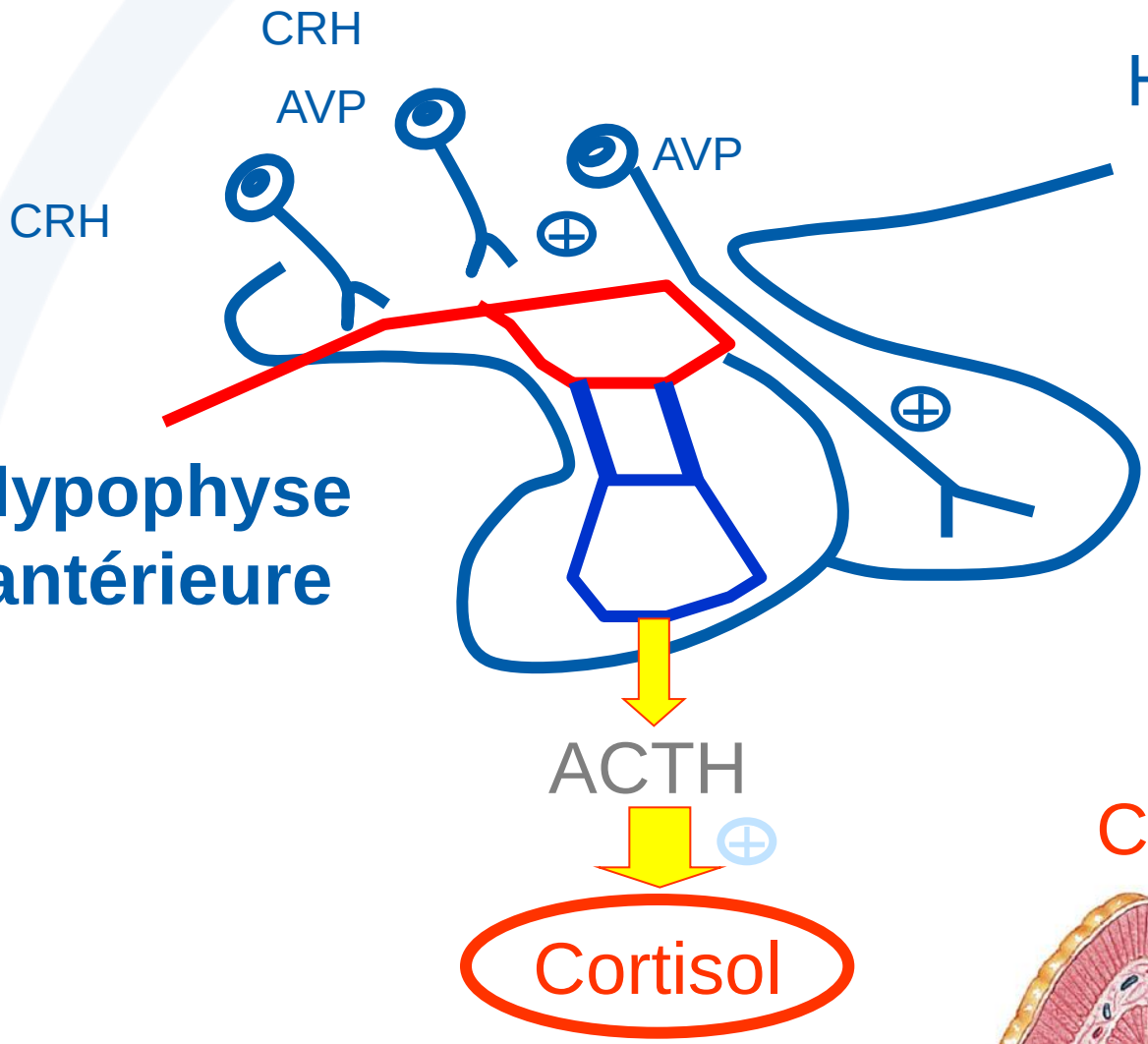


Exogenous Interferences

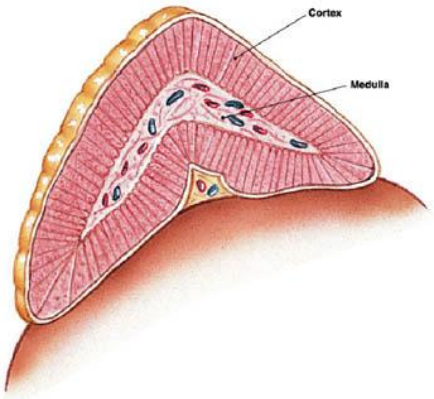
- Hemolysis, lipemia and icterus
- Matrix effects
- Blood collection tubes
- Label interferences
- Storage / Freeze-thaw
- Carry-over

Endogenous Interferences

- Excess antigen
- Antibody specificity
- Antibody interference
- Complements
- Paraproteins



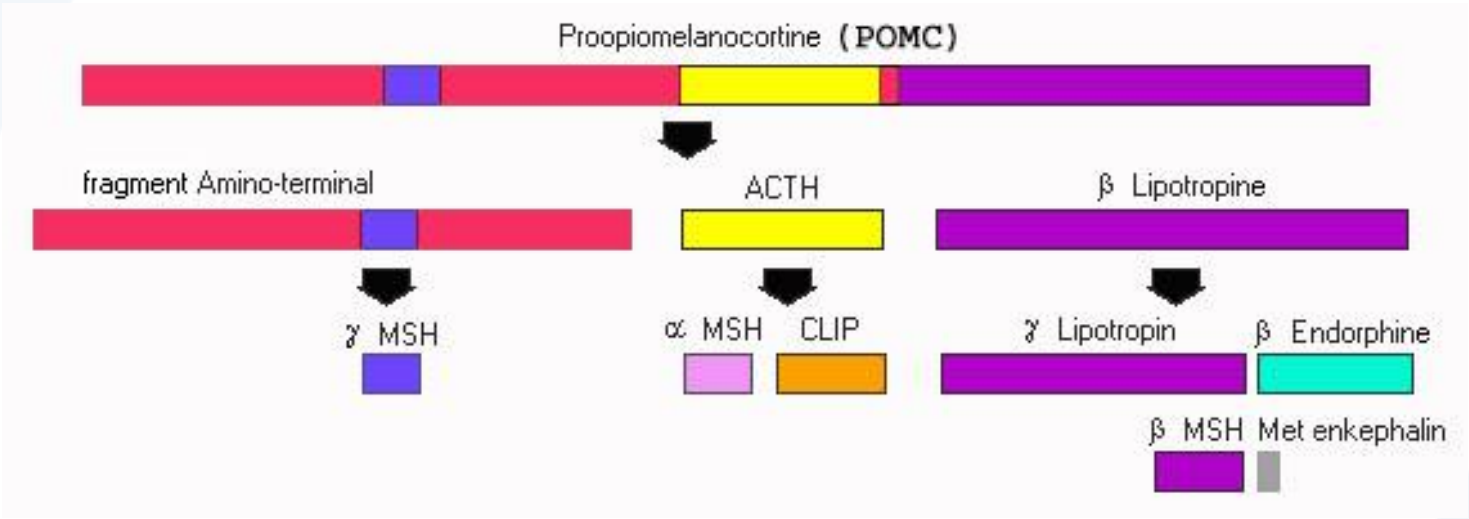
Corticosurrénales



Laboratory assays for Adrenal Hyperfunction and Adrenal Insufficiency

Disorder	ACTH	Cortisol	Dexamethasone Suppression
Adrenal insufficiency	↑	N or ↓	-
ACTH deficiency	N or ↓	↓	-
Adrenal Cushing	↓	↑	No
Pituitary Cushing	N or ↑	↑	Yes
Ectopic ACTH	↑↑↑	↑↑	Usually NO

ACTH testing

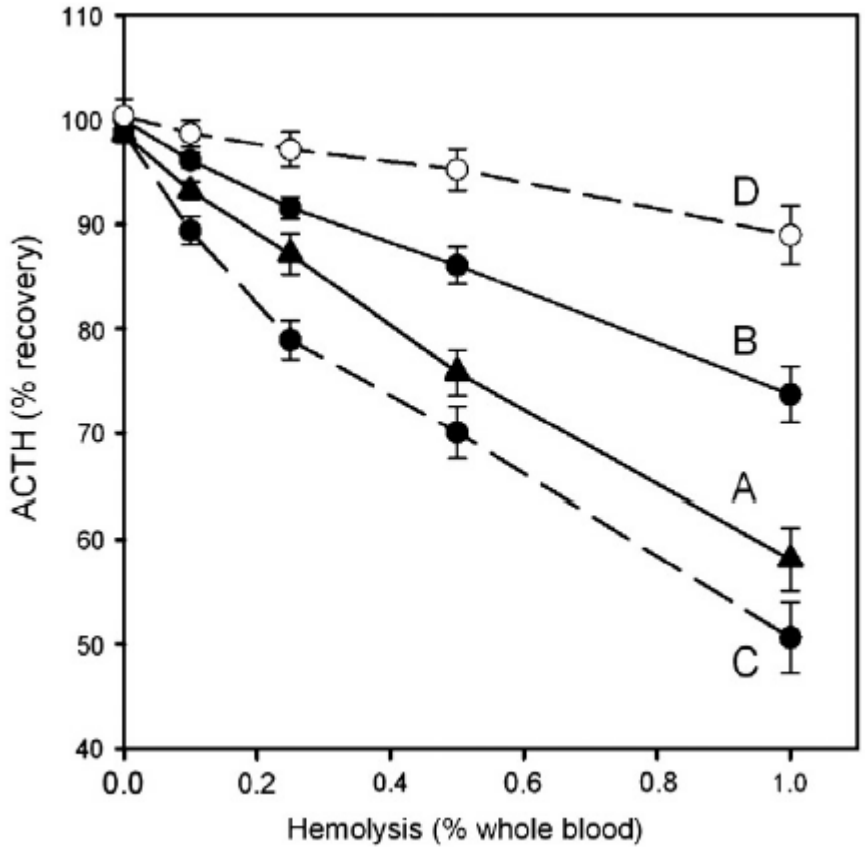


ACTH: 39 Amino acides

Molécule	Quantité ajoutée à l'échantillon, pg/mL	% réaction croisée
ACTH (1-10)	250 000	indéterminable
ACTH (1-24)	1 400	0,034
ACTH (18-39)	1 600	0,2
ACTH (11-24)	9 800	0,03
Alpha-MSH	3 000	0,04
Bêta-MSH	2 450	0,06
Bêta-endorphine	49 000	0,01
Somatostatine	9 800	0,09
Neurotensine	9 800	< 0,01
Enképhaline	9 800	< 0,01

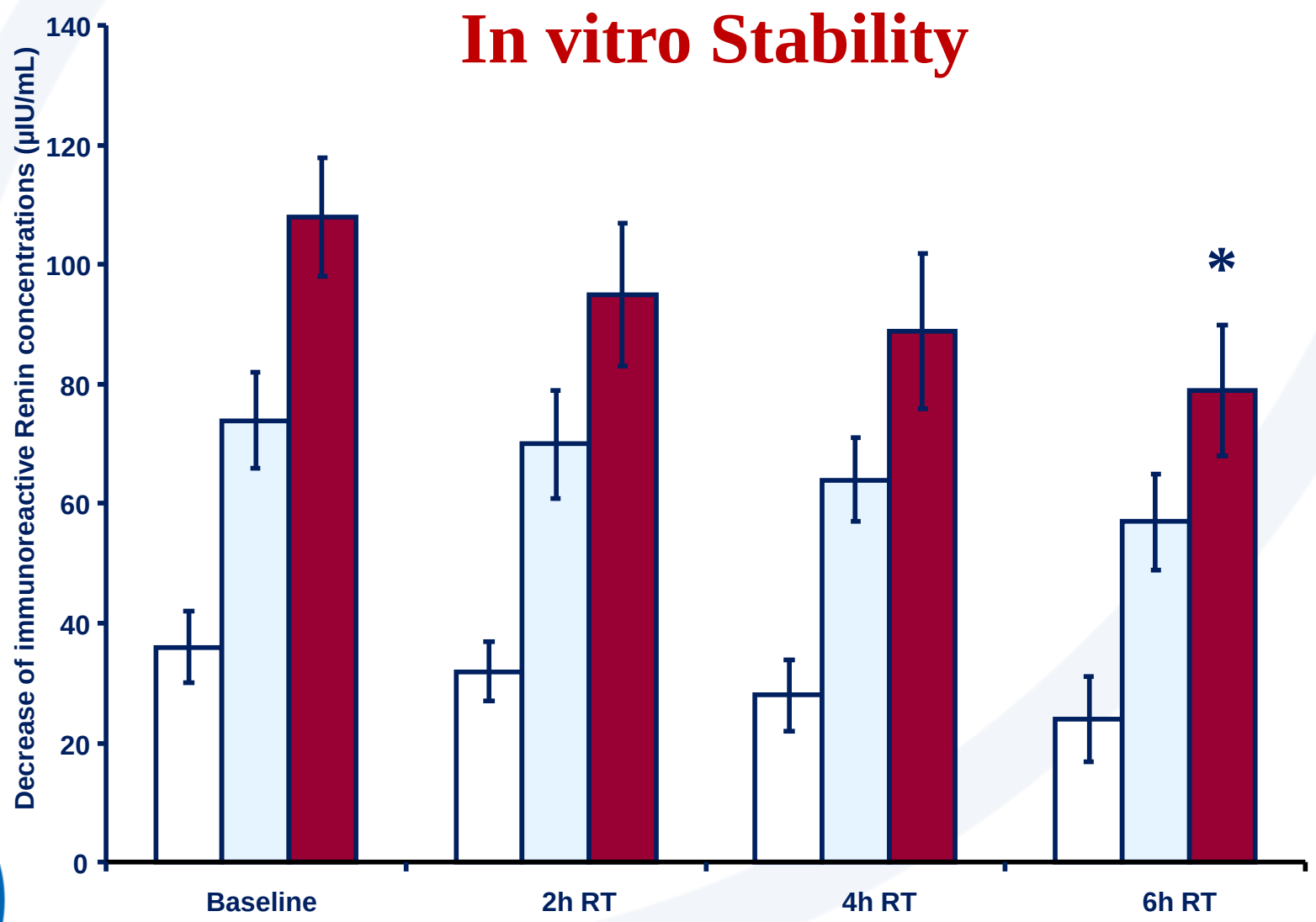
Table 1
Experimental design: summary of differences between sample treatments.

Treatment	Ice/water post-venepuncture	One hour 22 °C before analysis	NPM (2 mM)
A	−	−	−
B	+	−	−
C	+	+	−
D	+	+	+



Livesey et al. 2010

Direct / Active Assays: confounding factors



Cas Clinique 1

Monomeric prolactin (Mr 23 000)

- most common form of circulating prolactin in healthy individuals and most patients with true hyperprolactinemia

big prolactin (Mr 60 000)

big-big prolactin or macroprolactin [Mr 150 000)

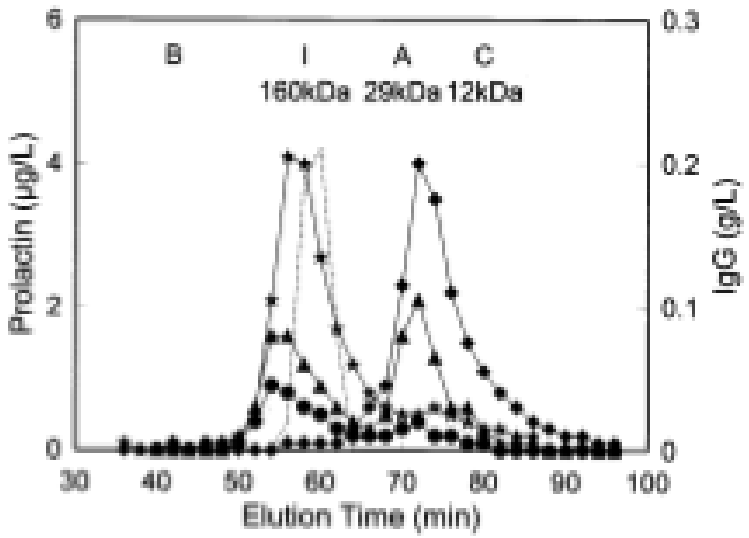


Table 2. Reference intervals for total prolactin (mIU/L) in serum samples from males and females for each immunoassay platform.

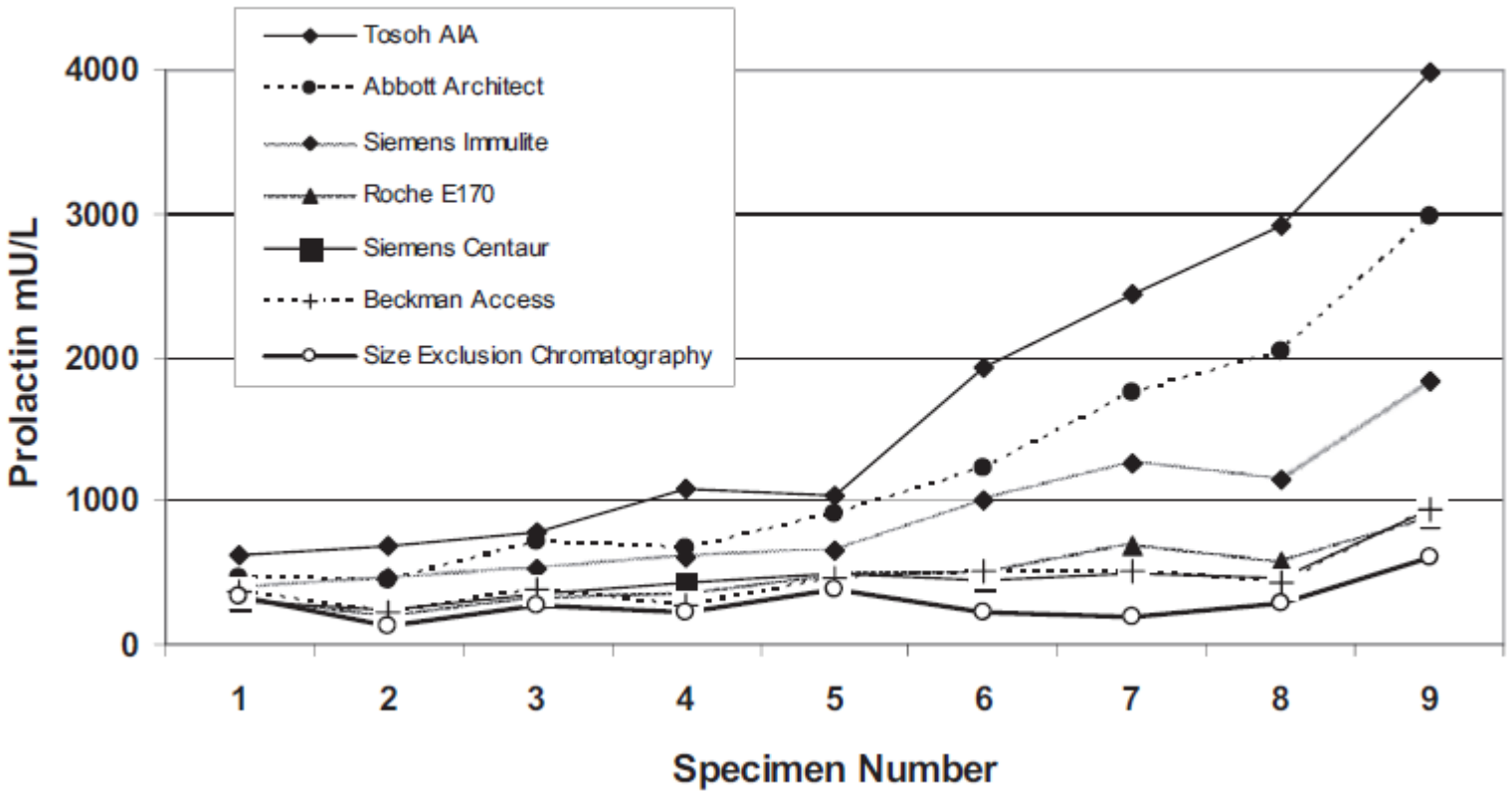
Method	Parametric lower	Estimate upper	Manufacturer's range
Samples from males			
Access	58	277	56–278
Centaur	63	262	45–375
Immulite	70	281	53–360
Elecsys	72	331	86–324
Architect	85	310	54–381
AIA	89	365	97–440
Samples from females			
Centaur	71	348	59–619
Immulite	75	396	40–530
Access	77	408	71–566
Elecsys	88	492	102–496
Architect	98	447	25–629
AIA	105	548	111–780

Clinical Chemistry 54:10
1673–1681 (2008)

Serum Total Prolactin and Monomeric Prolactin Reference Intervals Determined by Precipitation with Polyethylene Glycol: Evaluation and Validation on Common ImmunoAssay Platforms

Luisa Beltran,[1] Michael N. Fahie-Wilson,[1] T. Joseph McKenna,[2] Lucille Kavanagh,[2] and Thomas P. Smith[2*]

PROLACTIN



Mean prolactin levels obtained using six different prolactin methods in sera collected from nine macroprolactinaemic subjects. For comparative purposes the monomeric prolactin level in each specimen determined by size exclusion chromatography is shown.

Fahie-Wilson et al., 2013

PRL testing

250 ùL of serum

Mixed with an equal volume of PEG 6000, 250 g/L in PBS, pH 7.4

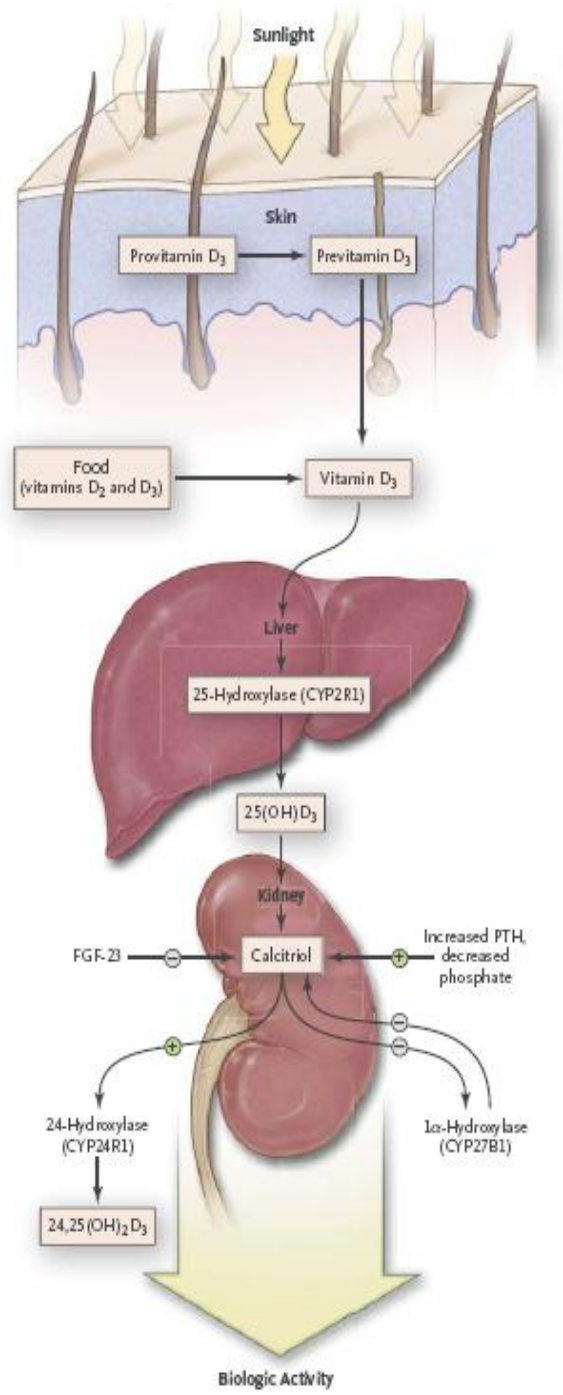
Incubated for 10 min at room temperature

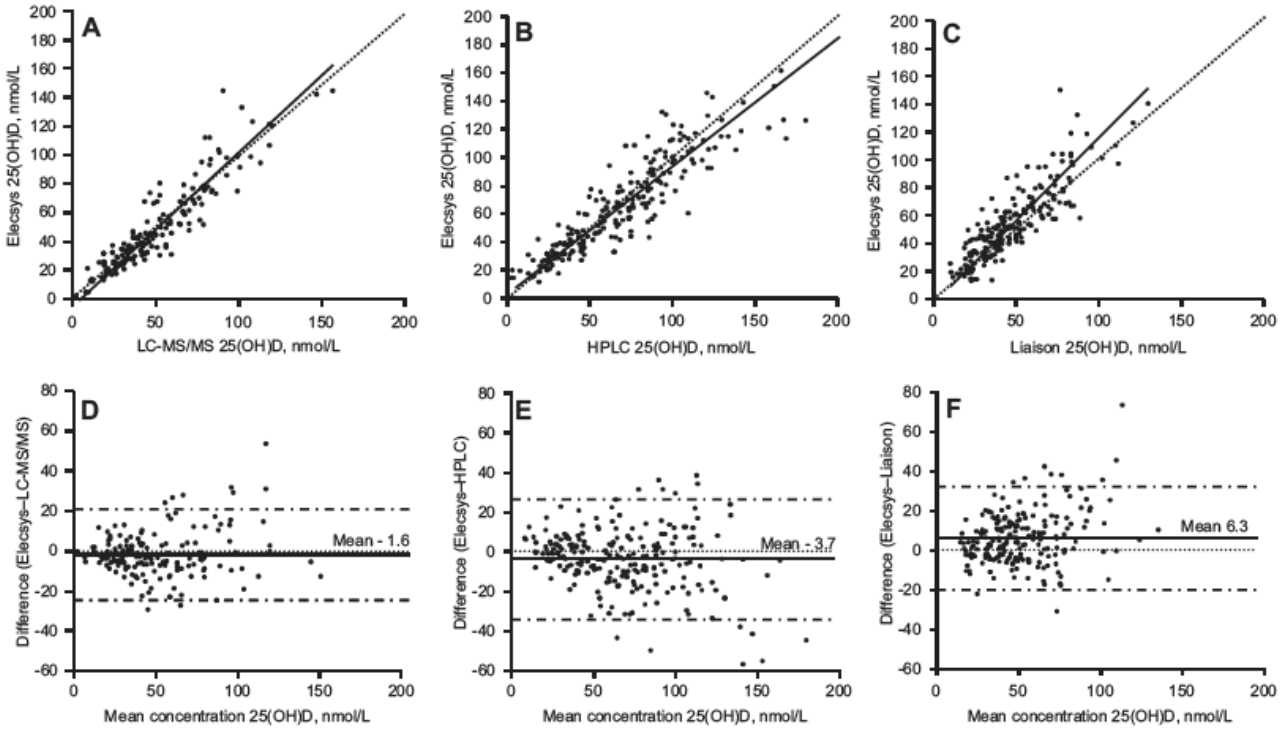
Suspension clarified by centrifugation at 14 000g for 5 min

Prolactin measurement.

Cas Clinique 2

VITAMINE D


Sunlight
Skin
Provitamin D3
Previtamin D3
Food
(vitamins D2 and D3)
Vitamin D3
Liver
25-Hydroxylase (CYP2R1)
25(OH)D3
Kidney
FGF-23
Calcitriol
Increased PTH,
decreased
phosphate
24-Hydroxylase
(CYP24R1)
1α-Hydroxylase
(CYP27B1)
24,25(OH)2D3
Biologic Activity

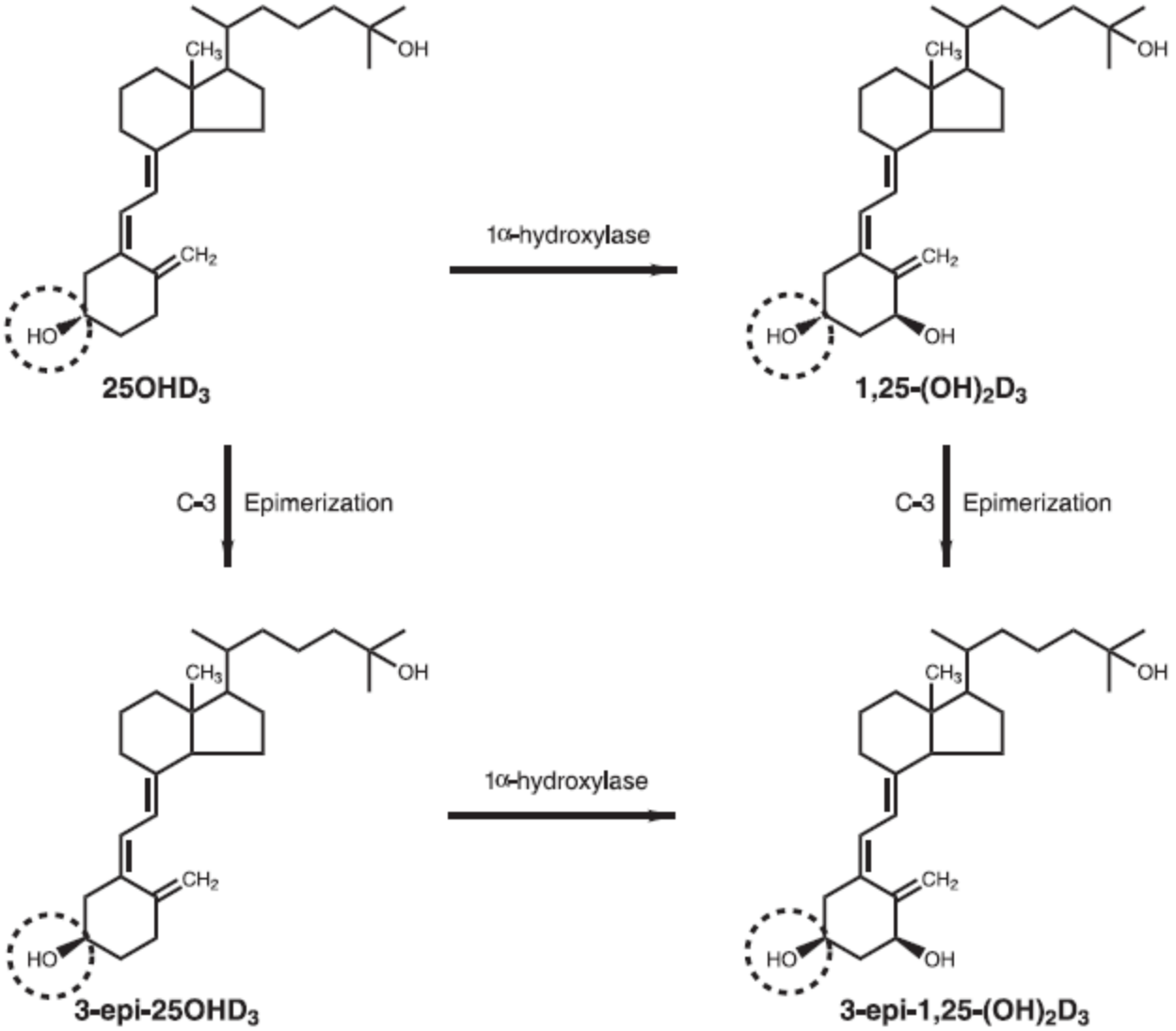


Emmen et al. 2012

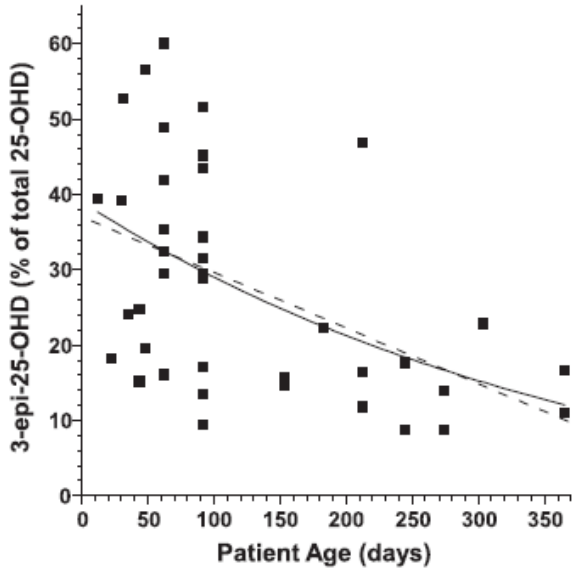
Table 1 Total and repeatability precision (inter- and intra-assay CV) per CLSI EP15-A2.

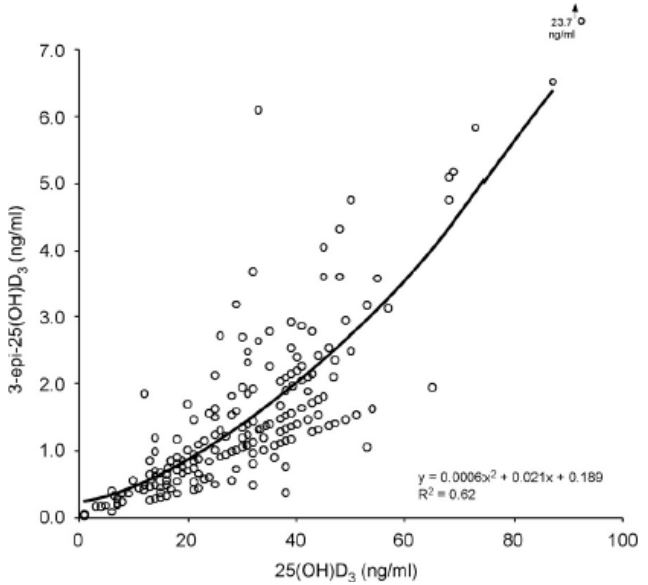
Specimen	LCMS	Abbott			DiaSorin			Roche			Siemens		
	Mean, nmol/L	Mean, nmol/L	Total CV	Repeatability CV	Mean, nmol/L	Total CV	Repeatability CV	Mean, nmol/L	Total CV	Repeatability CV	Mean, nmol/L	Total CV	Repeatability CV
MF1	3.0	16.5	30.1%	30.1%	5.9	25.4%	7.1%	<7.5	–	–	14.5	14.4%	11.9%
MF2	5.8	17.2	18.4%	18.4%	8.0	15.2%	4.9%	<7.5	–	–	17.5	13.3%	9.2%
MF3	12.8	20.3	23.6%	23.6%	10.3	10.7%	4.6%	<7.5	–	–	22.8	10.4%	10.4%
MF4	11.3	18.9	22.1%	10.9%	12.2	8.5%	6.3%	<7.5	–	–	19.4	12.4%	8.9%
MF5	17.5	22.1	13.5%	10.8%	18.5	6.7%	3.8%	19.4	24.5%	11.7%	16.9	10.5%	8.7%
MF6	24.3	24.2	13.2%	11.3%	24.1	5.5%	4.3%	24.4	18.8%	11.9%	20.0	8.7%	8.4%
MF7	40.0	32.9	8.6%	4.8%	32.1	7.8%	5.2%	34.8	18.7%	9.8%	24.9	10.9%	10.9%
MP1	34.8	44.3	9.3%	8.8%	37.2	5.2%	3.3%	50.6	11.7%	5.1%	29.6	8.7%	8.7%
MP2	63.8	56.4	6.2%	3.9%	59.5	5.5%	4.0%	54.7	12.6%	4.0%	45.9	20.3%	17.2%
MP3	86.5	56.4	12.1%	12.1%	85.7	4.0%	2.0%	98.3	8.3%	4.8%	59.3	10.3%	6.8%
MP4	257.5	178.5	5.8%	2.8%	176.6	6.2%	3.2%	152.8	4.0%	2.9%	216.2	4.7%	4.3%
MPS	370.3	282.6	4.3%	2.1%	>375	–	–	>175	–	–	317.4	3.1%	1.3%

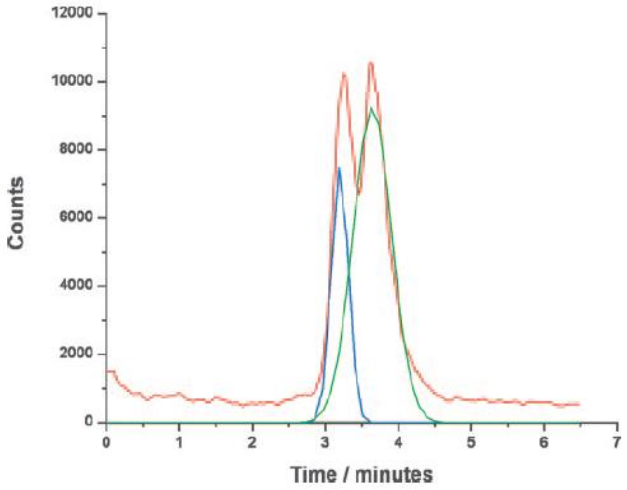
Farrell et al. 2012

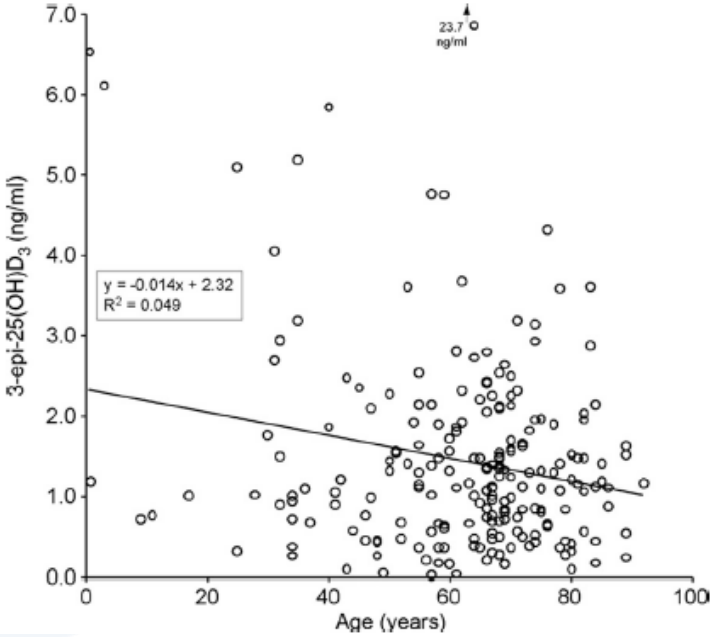


Singh et al.; 2006



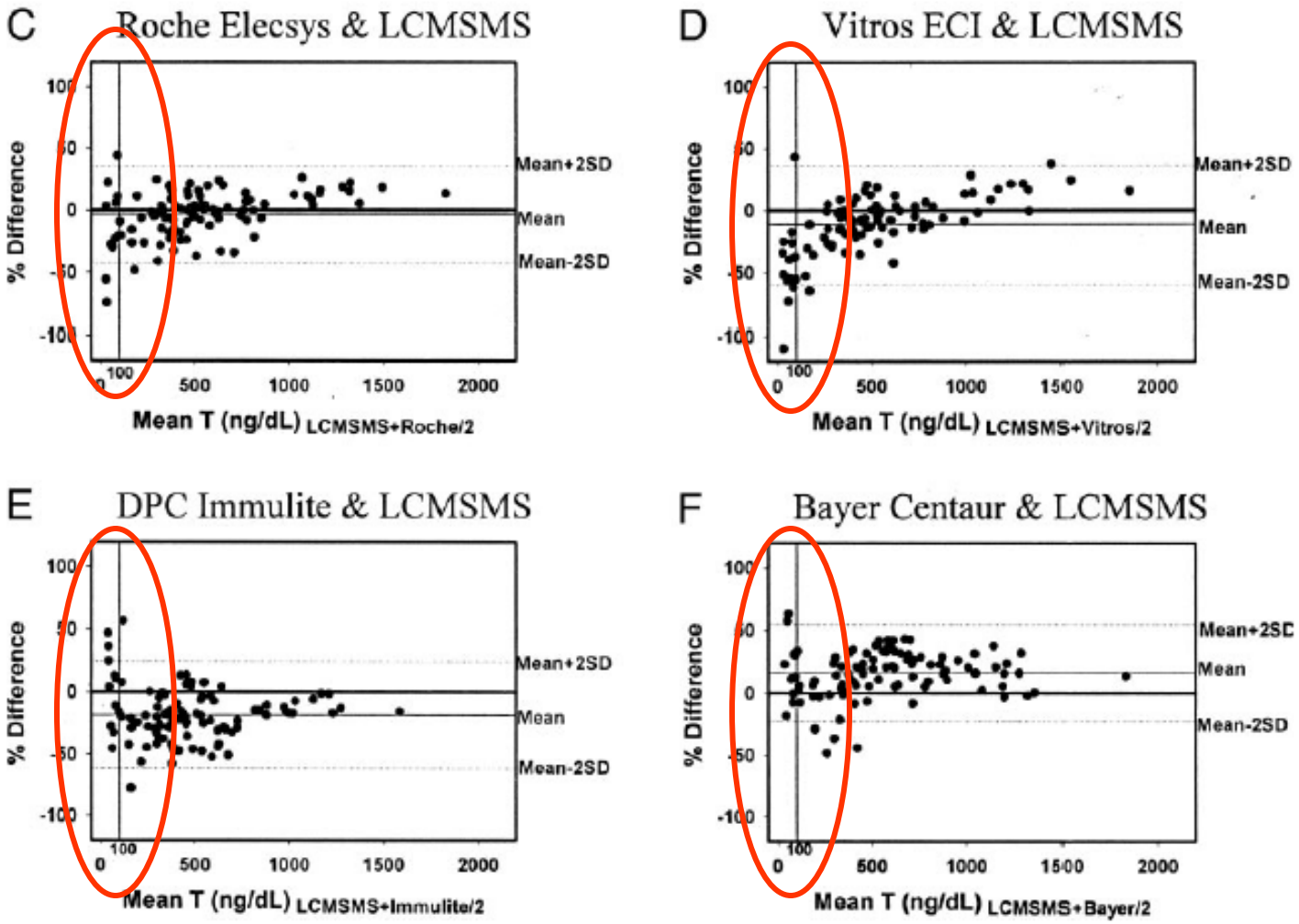






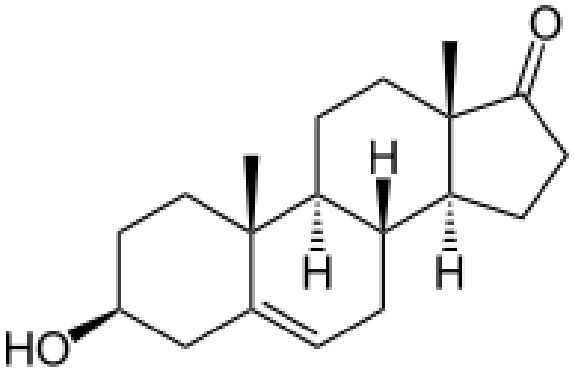
Singh et al.; 2006

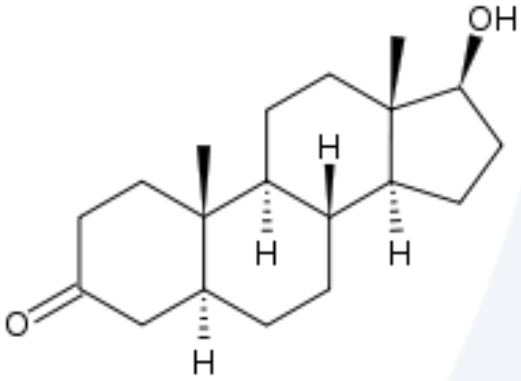
Testosterone assay

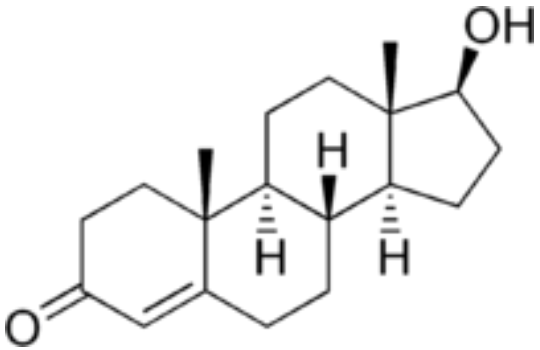


Wang et al., JCEM 2004

Testosterone assay







Ann Clin Biochem. 2007 Mar;44(Pt 2):173-7.

Dehydroepiandrostenedione sulphate interferes in many direct immunoassays for testosterone.

Middle JG.

UK NEQAS, Wolfson EQA Laboratory

Serum / Plasma cortisol testing

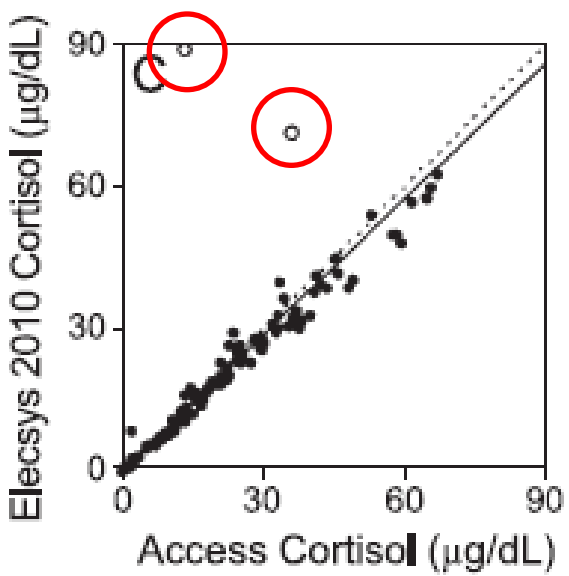
% CROSS-REACTIVITY	Immuno 1	Immunotech RIA	Elecsys E 170	Access Dxi	Centaur	DPC Immulite	DPC RIA
Prednisone	3.4	1	0.5	7.8	26.5	6.1	5.8
Fludrocortisone	1	27.5	6.8	8.3	5.4	< 0.1	4.4
Dexamethasone	< 0.1	< 0.1	< 0.1	1	0.2	< 0.1	< 0.1
Triamcinolone	< 0.1	< 0.1	< 0.1	0.7	< 0.1	< 0.1	< 0.1
Methylprednisolone	7.8	0.7	125.4	5	18.6	22	14
Cortisone	2.2	3.7	0.4	10.4	28	1	6.8
Prednisolone	55.8	11.2	68.1	43.8	31.5	62	67.8
Bethamethasone	< 0.1	< 0.1	< 0.1	1	< 0.1	< 0.1	< 0.1
20-alfa-dihydrocortisone	< 0.1	< 0.1	0.5	1	1.2	< 0.1	< 0.1
Flumethasone	< 0.1	< 0.1	< 0.1	0.6	0.1	< 0.1	< 0.1

Billen et al.

Serum / Plasma cortisol testing

Performance characteristics of five automated serum cortisol immunoassays

Richard F. Roberts[a,*] and William L. Roberts[b]



The reason for the two discordant results that were unique to the Elecsys 2010 method is unknown. It is most likely due to a cross-reacting substance(s) in the subjects' serum. This substance(s) could be either an endogenous or exogenous steroid. Additional information about these samples including age, gender, diagnosis, and medications is not available. When one considers endogenous substances, it is known that patients with 21-hydroxylase deficiency can exhibit elevated 21-deoxycortisol concentrations [10]. A review of assay package inserts revealed that the Elecsys 2010 cortisol method demonstrates 45.4% cross-reactivity with 21-deoxycortisol compared to the Advia Centaur method which only shows 4.5% cross-reactivity with this endogenous steroid (Table 3). Unfortunately, cross-reactiv-

Clin Biochem 2004

Salivary Cortisol testing

Table 1 Characteristics of the immunoassays (IAs) distributed by the respective manufacturers in 2008 and 2012.

Label	Type	Sales	Origin	IA cross-reactivities in 2008			IA cross-reactivities in 2012		
				Cortisone	17α-OHP	DEX	Cortisone	17α-OHP	DEX
IBL	CLIA	IBL International	Hamburg, Germany	3.2	0.5	<0.1	4.5	2.0	<0.1
DRG	EIA	DRG Instruments	Marburg, Germany	3.0	0.5	<0.1	3.0	0.5	<0.1
Salimetrics	EIA	Salimetrics	State College, PA, USA	0.3	0.6	1.3	0.1	<0.1	19.2
DSL	EIA	Diagn. System Lab.	Webster, TX, USA	7.0	0.9	0.9	–	–	–
DELFIA	EIA	In-house	Trier, Germany	0.6	NA	0.4	0.6	NA	0.4

Note: Distribution of the DSL kit has meanwhile been ceased after acquisition of Diagnostic System Laboratories by Beckman Coulter. Another available high sensitivity DRG kit features a higher cross-reactivity with cortisone (6.9%). CLIA, chemiluminescence IA; EIA, enzyme IA; 17α-OHP, 17α-hydroxyprogresterone; DEX, dexamethasone; NA, not available/not determined by manufacturer.

8-10 AM: 0.04 – 0.56 µg/dL
4-6 PM: <0.15 µg/dL
10-11 PM: < 0.09 µg/dL

Mass Spectrometry

Cortisol testing

Liquid chromatography and mass spectrometry

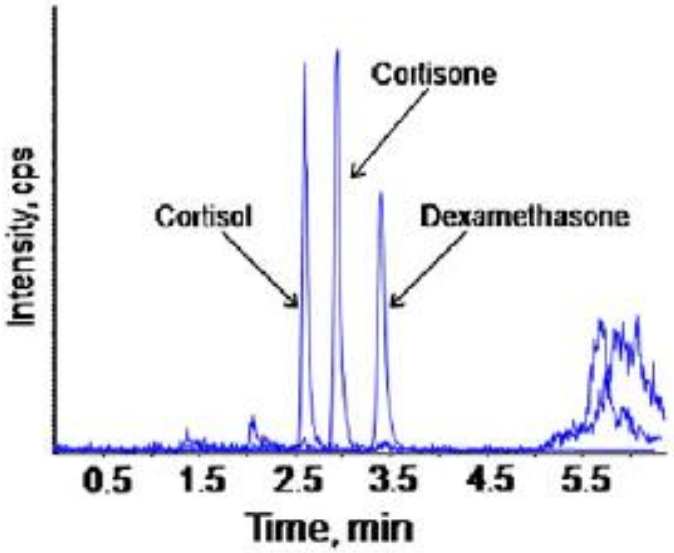


Table 1
Optimized mass spec parameters for 3 analytes and internal standards: M + 1 precursor and fragment ions.

Analyte internal standard	M + 1/fragment	DP(V)	EP(V)	CE(V)	CXP(V)
Cortisol	363/121	70	8	36	7
d4-cortisol	367/121	70	8	36	7
Cortisone	361/163	70	8	33	14
d8-cortisone	369/169	70	8	33	14
Dexamethasone	393/373	47	8	14	15
d4-dexamethaone	397/377	47	8	14	15

Declustering potential (DP), entrance potential (EP), collision energy (CE), and exit potential (CXP).

Kushnir et al.

VITAMINE D Measurement

The New York Times

Published: January 7, 2009

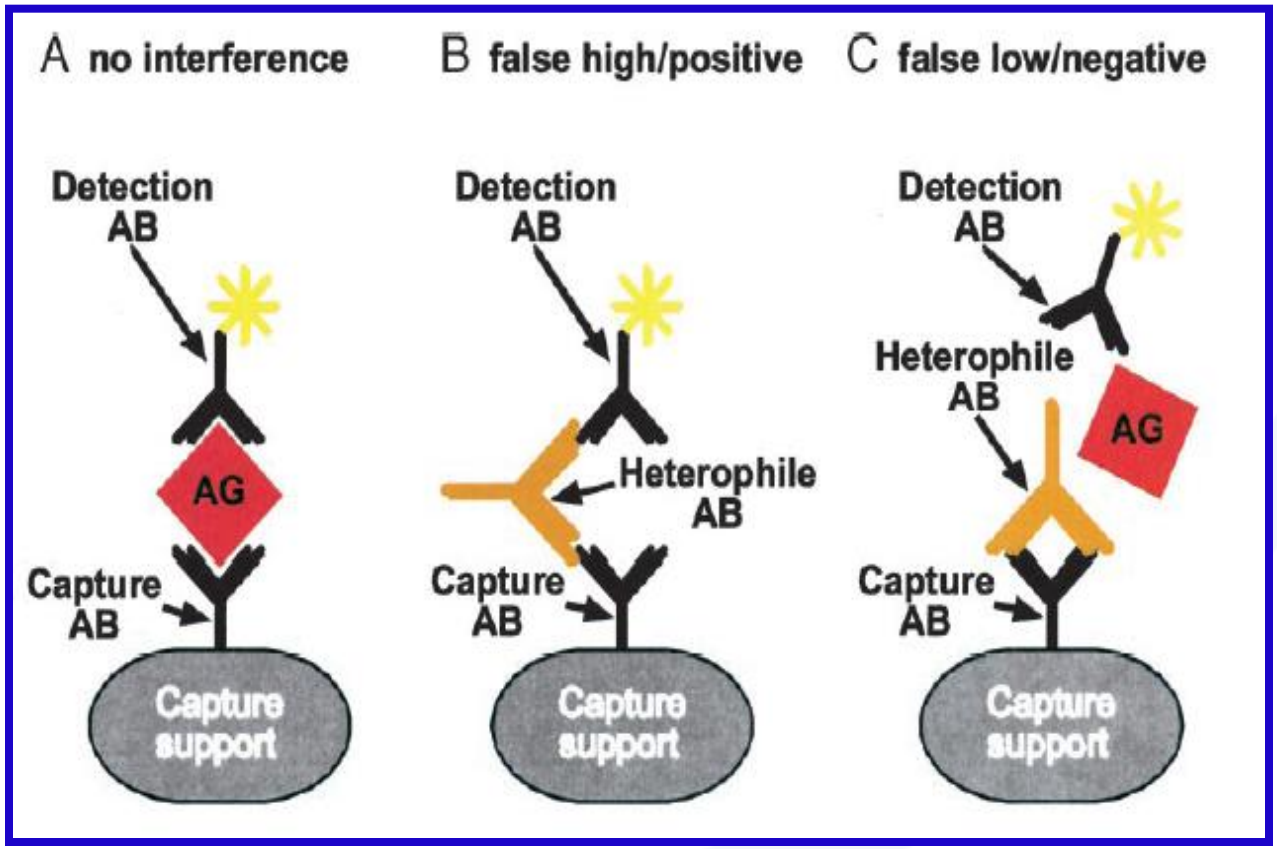
"Quest Acknowledges Errors in Vitamin D Tests"

Cas Clinique 3


Who you gonna call?
GHOSTBUSTERS
For professional paranormal investigation and eliminations

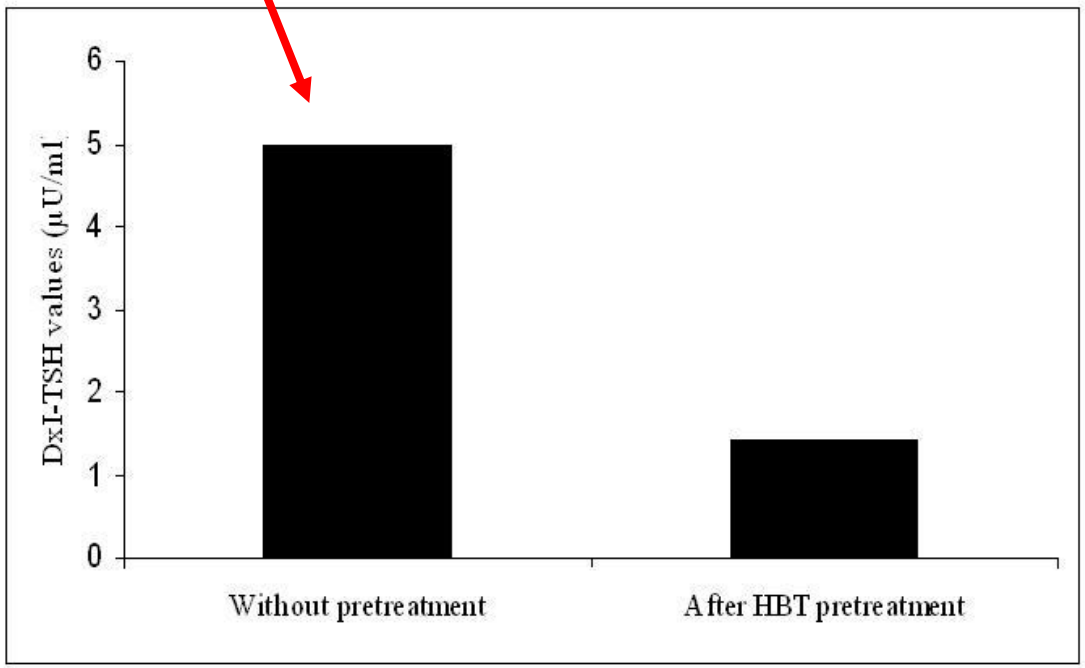
Phantoms in the Assay Tube: Heterophile Antibody Interferences in Serum Thyroglobulin Assays

CAROL M. PREISSNER, DENNIS J. O'KANE, RAVINDER J. SINGH, JOHN C. MORRIS, AND STEFAN K. G. GREBE



Preissner et al., J Clin Endocrinol Metab, 2003

TSH = 4.8 µUI/ml



E-05

Prevalence of Heterophile Antibody Interference with the Beckman Coulter, Inc. Access® TSH Assay

B. K. King, G. Klee, J. McDonald, N. Baumann. *Mayo Clinic, Rochester, MN,*

Background: Heterophile antibody, or human anti-animal antibody, interference is a known limitation of immunoassays. Prevalence of heterophile antibody interference has been reported to be between 1-2% in the general population, but most of these antibodies do not cause assay problems. It is difficult to detect heterophile interference

A196 2011 AACC Annual Meeting Abstracts

American Thyroid Association (ATA) 81st Annual Meeting

This coverage is not sanctioned by, nor a part of, the American Thyroid Association.

From Medscape Medical News

Antibody Interference Can Taint TSH Assays

Nancy A. Melville

Authors and Disclosures

Print This Share

November 3, 2011 (Indian Wells, California) — Interference from heterophile antibodies poses the risk of erroneous results on the commonly used Access thyroid-stimulating hormone (TSH) assay (Beckman Coulter), according to the results of a study presented here at the American Thyroid Association 81st Annual Meeting.

Read this article on Medscape's free mobile app. Download Now

Heterophile antibodies, or human antianimal antibodies, are believed to stem from such things as animal-derived pharmaceuticals and antibody therapies, and the incidence tends to be higher in patients who have received blood transfusions or vaccines or who have been exposed to animals.

Prevalence estimates for heterophile antibodies range from 1% to 80% of the population, according to lead author Nikola A. Baumann, PhD, director of clinical chemistry and specimen processing at the Mayo Clinic in Rochester, Minnesota, who presented the data.

"The wide discrepancy is related to how the antibody is identified," she said, adding that the interference from the antibodies can cause either false-positive or false-negative results.

Dr. Baumann said that from 2008 to 2009, the Mayo Clinic's Endocrinology Clinic received about 20 to 30 requests per year from clinicians suspecting heterophile antibody interference on the Access TSH assay; the interferences would be confirmed in about a third of the samples. In 2010, however, a noticeable increase in antibody interference prevalence emerged.

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Clinica Chimica Acta 379 (2007) 171–172

Letter to the Editor

False-positive serum chromogranin A assay due to heterophile antibody interference

Circulating chromogranin A (CgA) measurement is a reliable tool in diagnosis and management of neuroendocrine tumours (NET). It's sensitivity widely range according to

buffers as HAb block
diagnosis, manageme
However, in our pa
might have been caus
mouse immunoglobul
As far as our knowlec

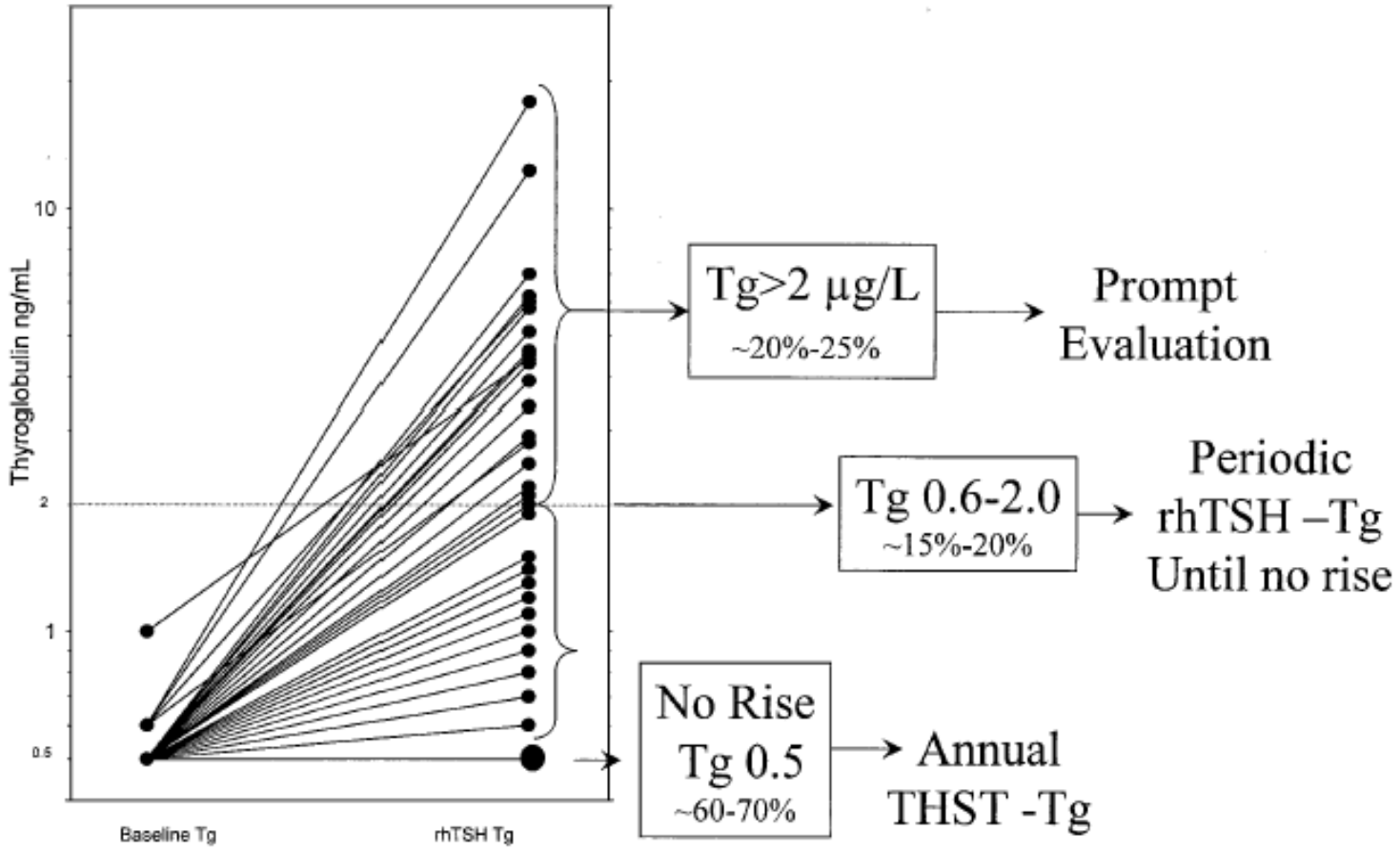
« Many recent studies showed 1–3% overall incidence of HAb-induced artefacts on different immunoassays for TSH, troponin, CA 125, thyroglobulin and many others analytes »

Tg testing

- Tg is a large glycoprotein comprising two apparently identical polypeptide chains (660 kDa).
- Stored in the follicular colloid of thyroid gland.
- Acts as pro-hormone in the intra-thyroid synthesis of thyroxine (T4) and triiodothyronine (T3)produced only by normal thyrocytes or well-differentiated thyroid cancer (DTC) cells.
- Serum Tg is elevated in patients with goiter and in most hyperthyroid conditions.

- Serum Tg concentrations reflects primarly 3 factors
  - ❖Mass of differentiated thyroid tissue present
  - ❖Any physical damage to or inflammation of the thyroid gland
  - ❖Magnitude of the TSH receptor stimulation.

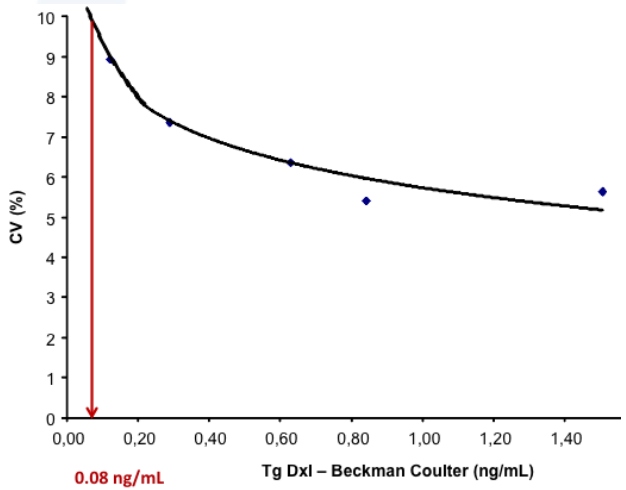
Tg testing

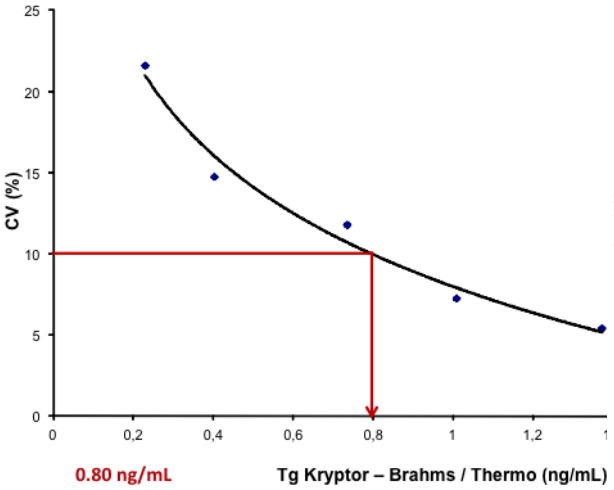


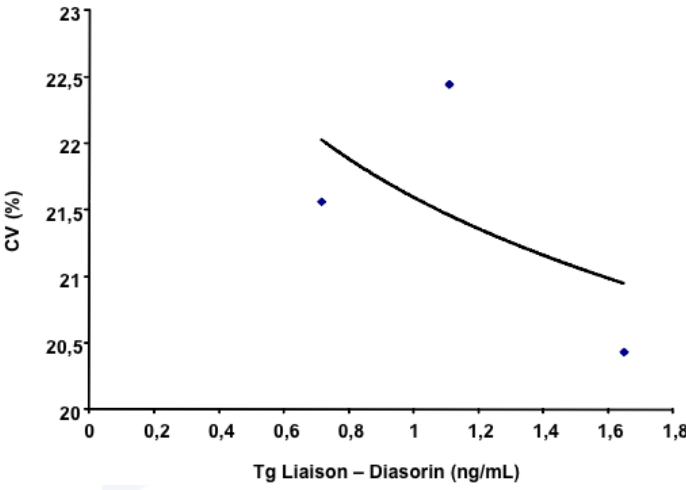
Mazzaferi et al., 2002

TABLE 1. Tg standard CRM 457 was diluted in human Tg free serum and then measured with each method

Serial dilutions of CRM 457 (ng/ml)	Calculated values (ng/ml)						
	Tg-Kryptor	Immulite TG	Thyro	Tg Access	Tg Advantage	DYNOtest	e-Iason TgCa
200	95	188	245	185	112	100	126
100	52	81	125	95	55	50	58
20	9.6	14.6	24.1	17	13.2	10	10.5
5	2.9	3.8	7.6	4.4	3.7	2.8	2
1	0.8	0.8	1.8	1	1.2	0.7	0.4
0.5	0.2	<0.2	1.4	0.5	0.5	0.2	0.2
0.25	<0.17	<0.2	0.91	0.33	<0.3	<0.1	0.13
0.10	<0.17	<0.2	<0.2	0.20	<0.3	<0.1	0.07







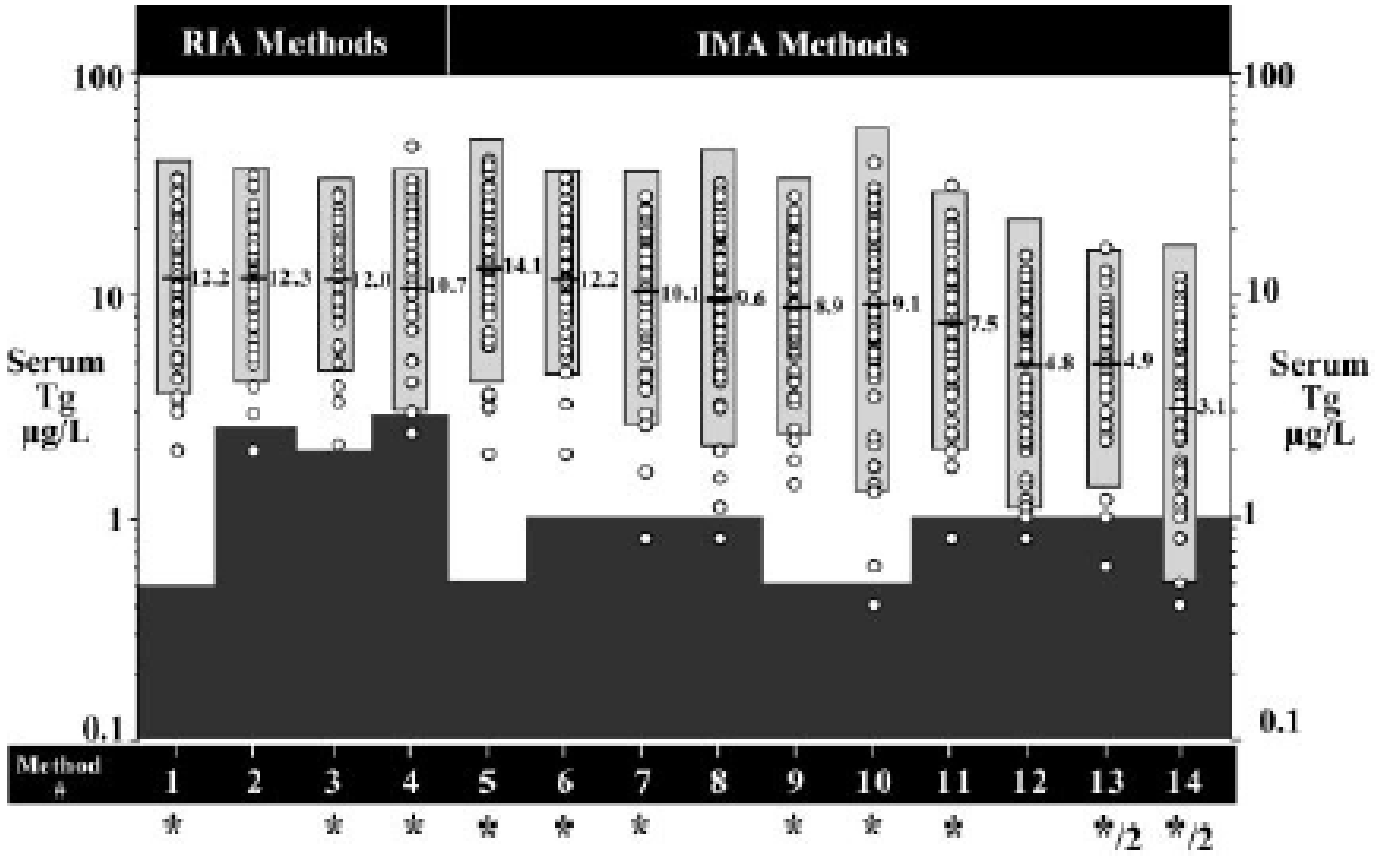
- sur 51 échantillons <0,2 ng/mL (indétectables) par RIA
- **9 échantillons** sont > 0,2 ng/mL par hTgs (détectables) → **17%**

RIA	Kryptor	Anti Tg	Anti TPO	Anti TSH	Recouvrement	CLINIQUE
0,2	0,8399	**29,61**	74,6	0,6	normal	**Thyroïdite auto-immune** et polyadénopathies cervicales
0,2	0,4618	/	/	15,7	**anormal**	**Suivi d'une maladie de Basedow.**
0,2	0,3361	/	/	/	normal	**Thyroïdectomie totale** le 28 novembre 2013 un traitement complémentaire par radioiode I-131 (100 mCi) sous Thyrogen en date du 15 janvier 2014.
0,2	0,3047	/	/	/	normal	**Thyroïdectomie totale** avec curage central en zone VI en date du 15 novembre 2013 La scintigraphie post-thérapeutique a mis en évidence une fixation du radioiode en région cervicale médiane ainsi qu'une seconde fixation plus marquée en cervical
0,2	0,2788	**9,2**	/	/	normal	**Thyroïdectomie totale** le 24/10/13 18/12/13 a reçu sous Thyrogen, un traitement complémentaire par radioiode 131 (100 mCi)
0,2	**0,2076**	1	/	/	normal	**Thyroïdectomie totale** 5/06/2013, suivi 6 mois après
0,2	**0,3947**	1,1	/	/	normal	//
0,2	0,2295	3	/	/	normal	**Carcinome papillaire** lobectomie thyroidienne gauche
0,2	0,3078	0,9	/	/	normal	**Thyroïdectomie totale** le 28 novembre 2013 traitement complémentaire par radioiode I-131 (100 mCi)

Clinical Impact of Thyroglobulin (Tg) and Tg Autoantibody Method Differences on the Management of Patients with Differentiated Thyroid Carcinomas

C. A. Spencer, L. M. Bergoglio, M. Kazarosyan, S. Fatemi, and J. S. LoPresti

antiTg Negative

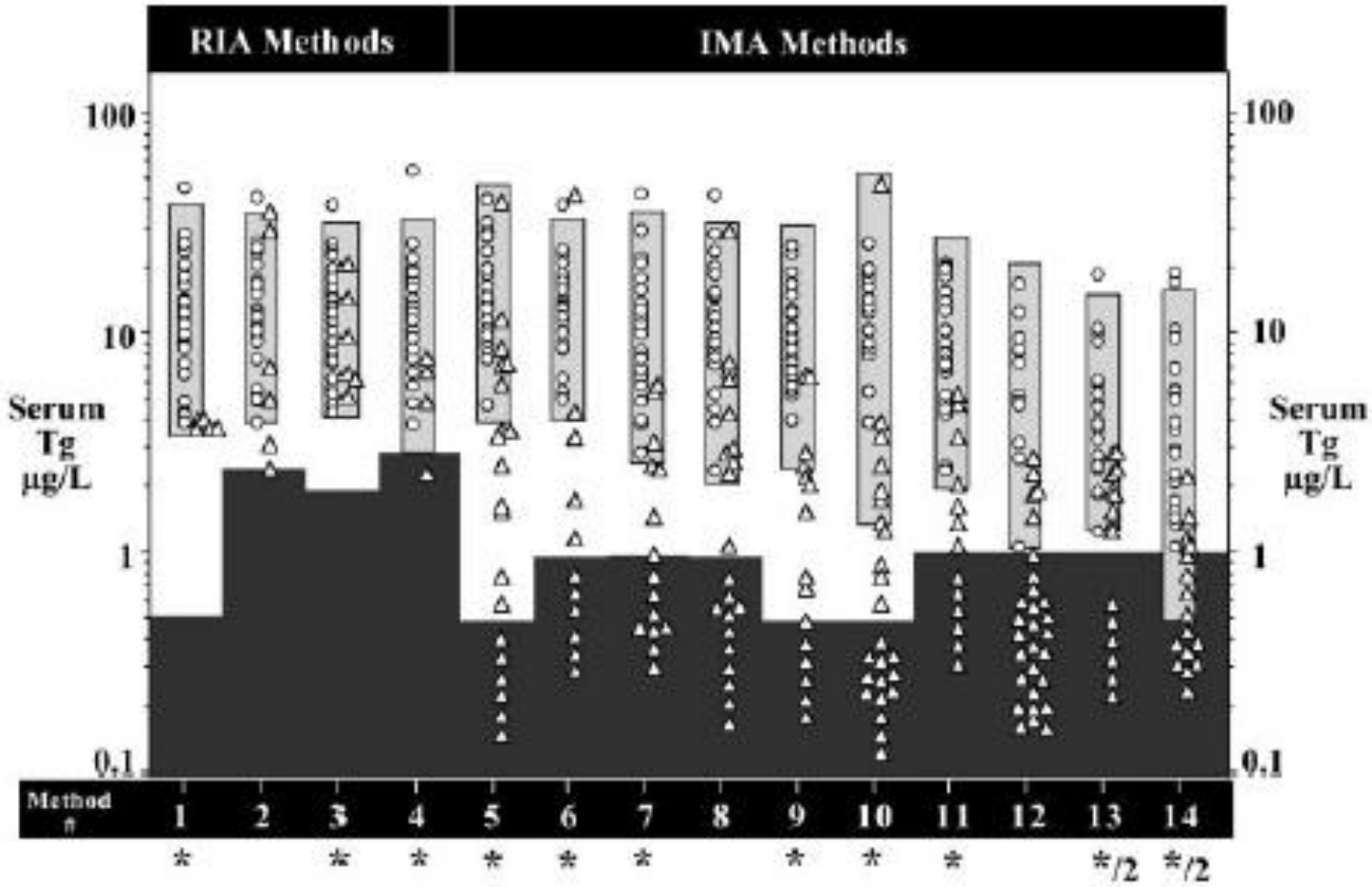


JCEM 2005

Clinical Impact of Thyroglobulin (Tg) and Tg Autoantibody Method Differences on the Management of Patients with Differentiated Thyroid Carcinomas

C. A. Spencer, L. M. Bergoglio, M. Kazarosyan, S. Fatemi, and J. S. LoPresti

antiTg Positive



JCEM 2005

Cas Clinique 4

Insulin testing and Hemolysis

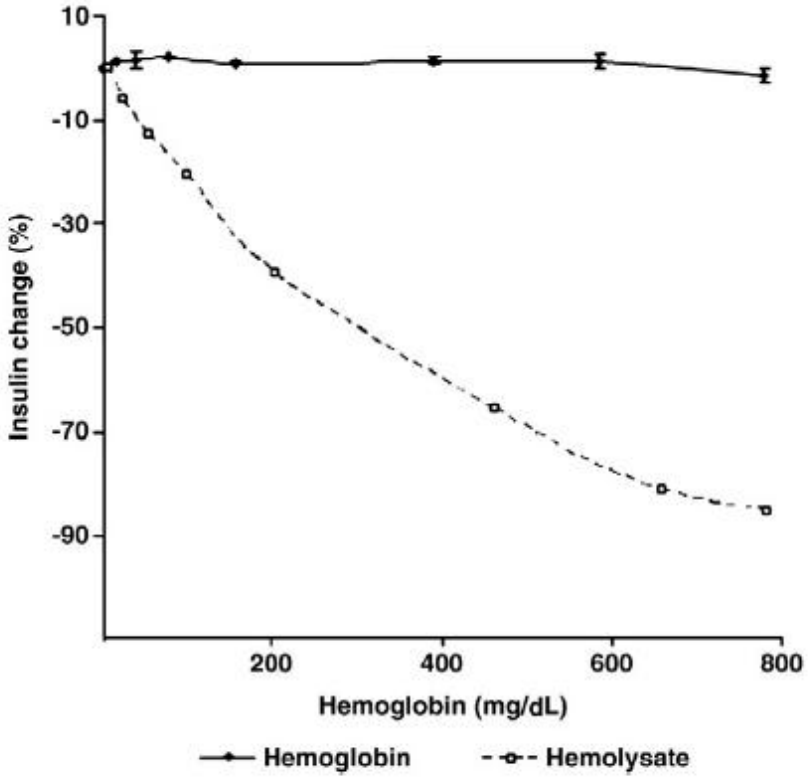
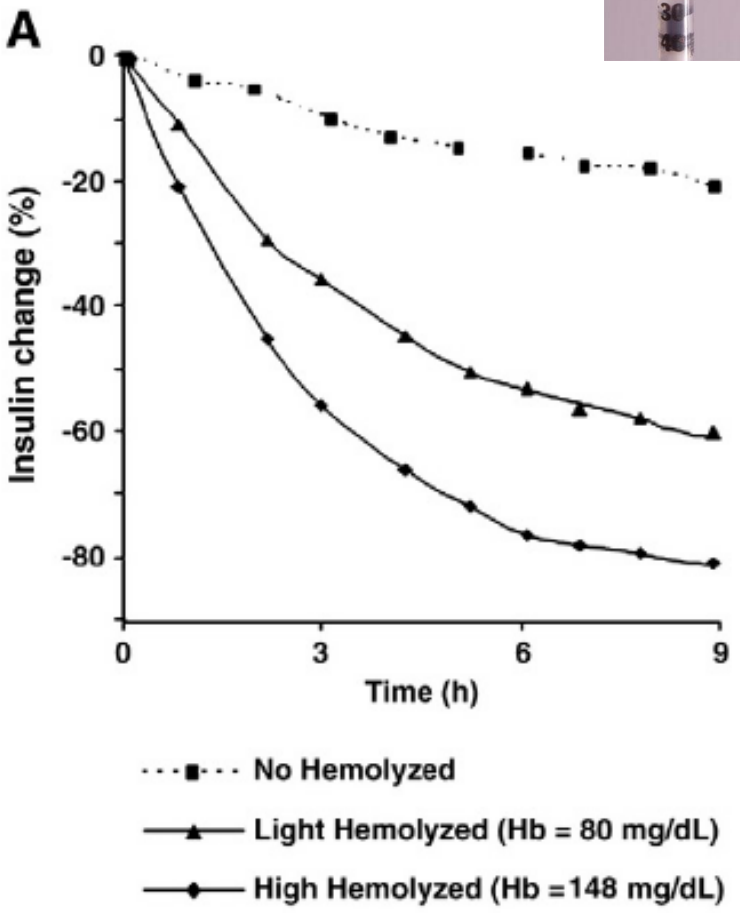


Fig. 1. Effect of hemoglobin and hemolysis on apparent insulin levels.



Garinet et al.; 2014

Insulin testing and Analogs

Table 1. Percentage cross-reactivity from the ratio of measured and nominal concentrations of insulin analogs.

	Cross-reactivities, %	
Insulin product	**ARCHITECT Insulin**	**E-test TOSOH (IRI)**
Aspart (NovoRapid)		
10 mIU/L	76	115
100 mIU/L	75	116
Glargine (Lantus)		
10 mIU/L	105	16
100 mIU/L	83	22
Lispro (Humalog)		
10 mIU/L	110	116
100 mIU/L	100	106

Table 2
Comparison of measured insulin concentrations between the Architect and ECLusys assays.

Insulin	Theoretical value (mU/l)	Architect (mU/l)	Recovery ratio (%)	ECLusys (mU/l)	Recovery ratio (%)
Regular human insulin	10,000	>300	–	>1000	–
	100	89.1	89	102.6	103
	10	10.4	104	10	100
NPH insulin	10,000	>300	–	>1000	–
	100	108.4	108	113.7	114
	10	10.0	100	11.53	115
Insulin lispro	10,000	>300	–	<0.2	0
	1000	>300	–	<0.2	0
	100	123.6	124	<0.2	0
	10	10.2	102	<0.2	0
Insulin aspart	10,000	>300	–	0.699	0.006
	1000	>300	–	<0.2	0
	100	84.4	84	<0.2	0
	10	10.0	100	<0.2	0
Insulin glargine	10,000	>300	–	<0.2	0
	1000	>300	–	<0.2	0
	100	105.3	105	<0.2	0
	10	10.7	107	<0.2	0

NPH, neutral protamine Hagedorn.

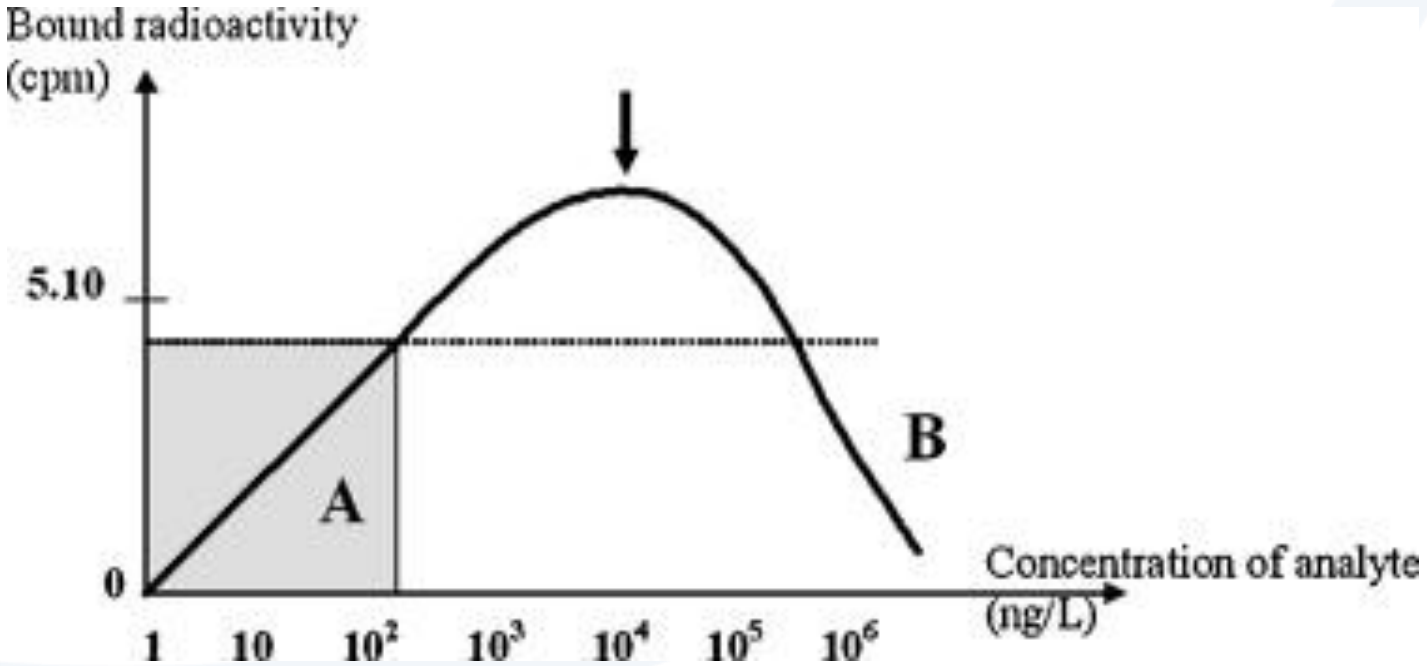
Table. Cross-reactivities of the Liaison® Insulin assay with Insulin Analogs (Ratio between measured and nominal concentrations)

	Cross-reactivities (%)	
Insulin Product	**Dilutions in BSA**	**Dilutions in Insulin depleted serum**
Glargine (Lantus®)		
10 mIU/L	5.2%	3.8%
100 mIU/L	12.7%	10.3%
500 mIU/L	36.3%	31.4%
Lispro (Humalog®)		
10 mIU/L	< 0.5%	< 0.5%
100 mIU/L	< 0.5%	< 0.5%
500 mIU/L	< 0.5%	< 0.5%
Aspart (NovoRapid®)		
10 mIU/L	< 0.5%	< 0.5%
100 mIU/L	< 0.5%	0.8%
500 mIU/L	0.7%	1.1%

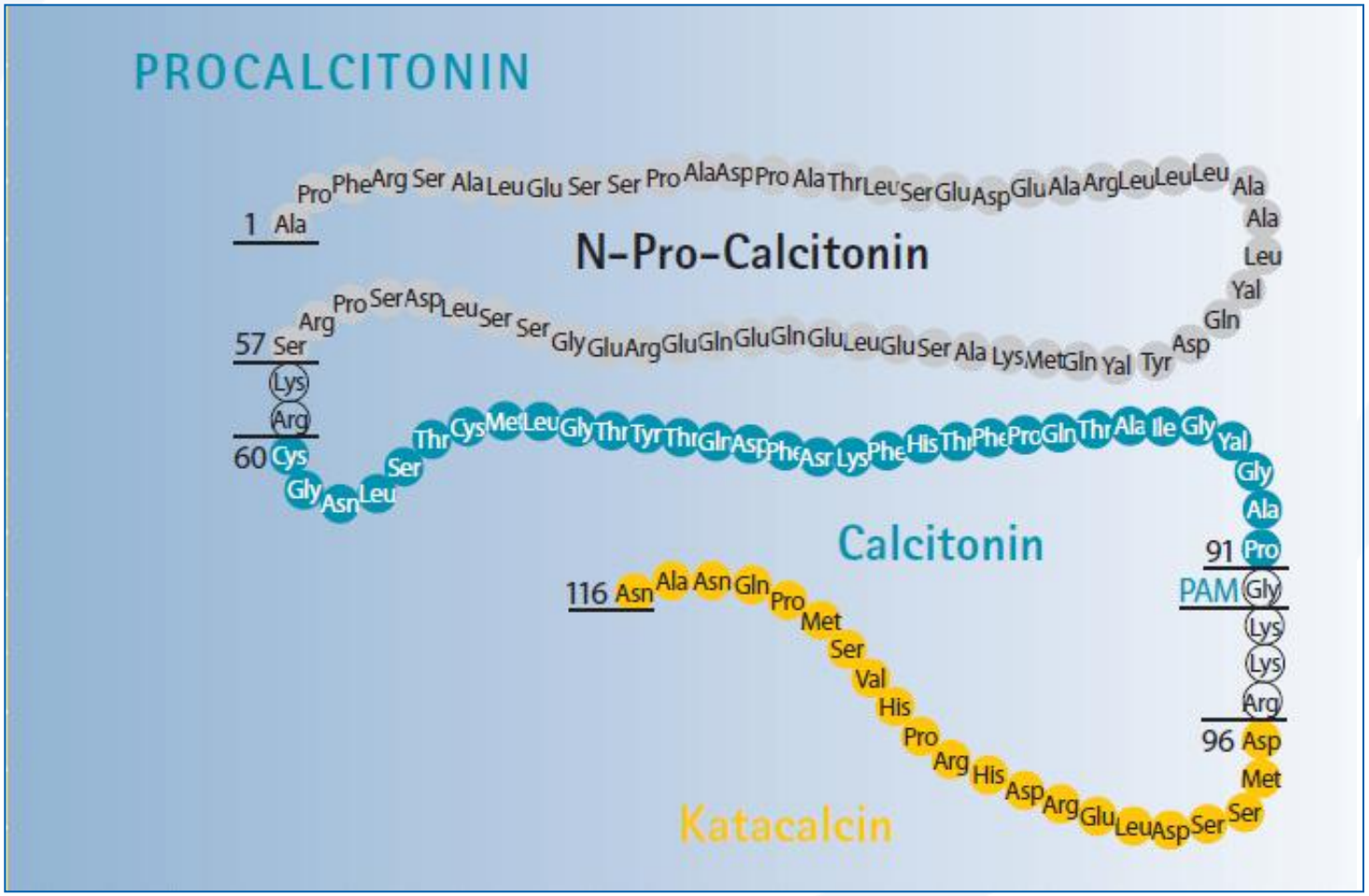
CA ME LAISSE PERPLEXE

Cas Clinique 5

« The Hook Effect »



Calcitonin testing



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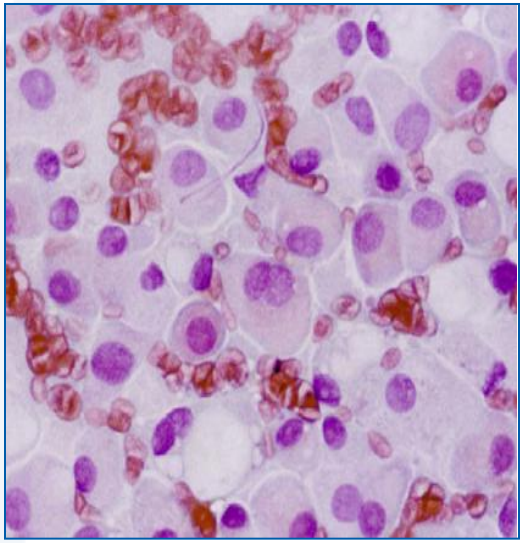
Medullary thyroid carcinoma (MTC)



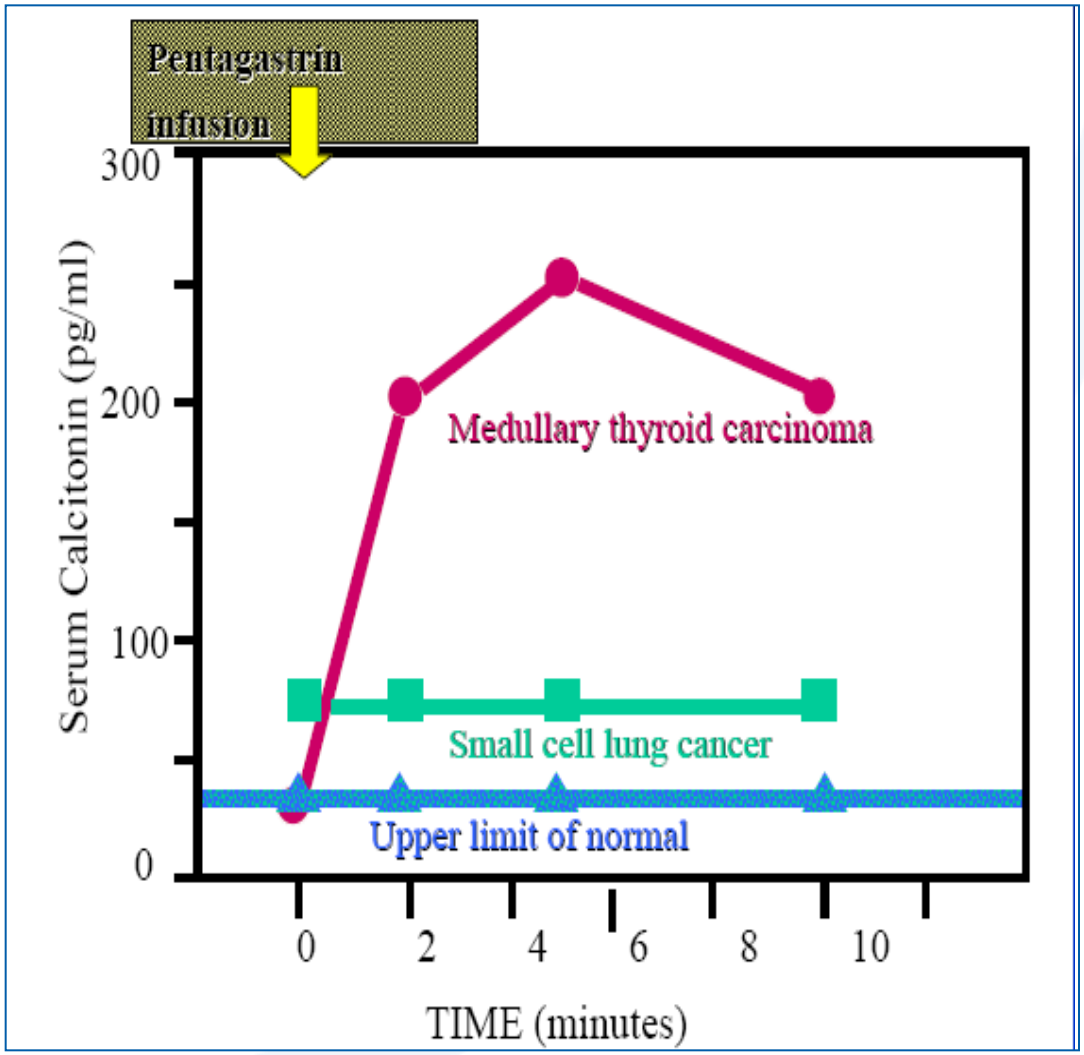
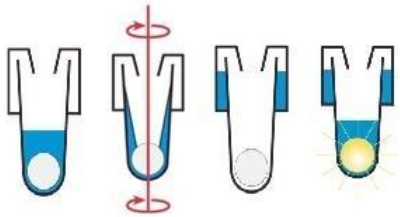


TABLE 1. Patient's serum calcitonin concentrations after several dilutions, using the DSL-7700 IRMA

Dilution	Calcitonin (ng/liter)
September 2004	
None	48
1:5	205
1:10	342
1:20	546
1:50	702
1:100	619
1:200	662
October 2004	
None	40
1:5	227
1:10	356
1:20	550
1:50	729
1:100	673
1:200	633
November 2004	
None	99
1:2	206
1:5	394
1:10	638
1:20	871
1:50	1427
1:100	1904
1:200	900

	**Advantage^{R} Nichols**	-Chemoluminescence (ester d'acridium) -41 min -Ac 11-23 et 21-32
	**Liaison^{R} DiaSorin**	-Chemoluminescence (isoluminol) -45 min -Ac ?
	**Immulite^{R} Siemens**	-Enzymatique (PA)  -2 x 30 mn

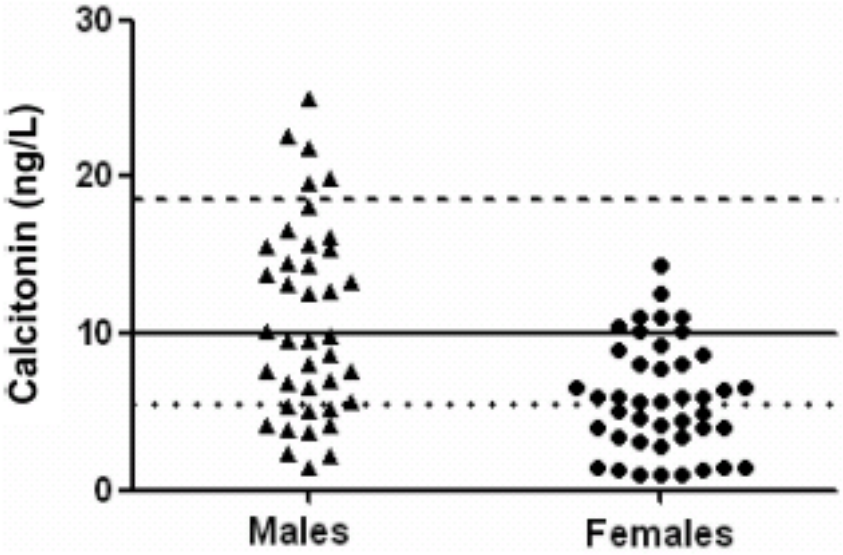


Fig. 1. Basal serum calcitonin concentrations in males (n = 40) and females (n = 43).

Indicated are the current diagnostic limit of 10 ng/L (solid line) and the manufacturer's upper reference limits for males (dashed line) and females (dotted line).

quand faire un enfant devient
un rendez-vous médical,

Table 1
Patient serum samples with markedly elevated β-hCG performed on the Beckman Coulter DxI800 and Abbott i-STAT. The i-STAT should display numerical values exceeding 2000 IU/L as ">2000 IU/L". Values exceeding 1000 IU/L will automatically be run in dilution on the Beckman Coulter DxI800.

Patient sample	Clinical diagnosis	Gestational age (weeks)	Beckman coulter DxI800 (IU/L)	Abbott i-STAT (IU/L)	Interpretation of reported i-STAT result
1	Ectopic pregnancy	7	2846	>2000	Correct
2	Ectopic pregnancy	6	18,000	>2000	Correct
3	Ectopic pregnancy	6	21,194	>2000	Correct
4	Miscarriage	6	22,826	>2000	Correct
5	Miscarriage	8	140,229	>2000	Correct
6	1st Trimester bleed	9	210,000	>2000	Correct
7	Hyperemesis gravidarum	10	240,000	>2000	Correct
8	Abdominal pain in pregnancy	10	250,000	>2000	Correct
9	Hyperemesis gravidarum	12	260,000	>2000	Correct
10	Hyperemesis gravidarum	14	290,000	1816	Hook effect
11	1st Trimester bleed	10	310,000	>2000	Correct
12	1st Trimester bleed	8	320,000	>2000	Correct
13	Gestational trophoblastic disease	–	360,000	1421	Hook effect
14	Hyperemesis gravidarum	13	390,000	1805	Hook effect
15	Gestational trophoblastic disease	–	520,000	1195	Hook effect
16	Gestational trophoblastic disease	–	1,200,000	717	Hook effect

Table 2
Dilutions of a serum sample with a markedly elevated β-hCG analysed on the DxI800 and i-STAT.

Dilution (serum:diluent)	Beckman Coulter DxI800 (IU/L)	Abbott i-STAT (IU/L)	Interpretation of reported i-STAT result
Neat	520,000	1195	Hook effect
1:1	277,265	1187	Hook effect
2:3	218,712	1702	Hook effect
1:4	117,000	>2000	Correct
1:150	4514	>2000	Correct
1:200	3344	>2000	Correct
1:250	2705	>2000	Correct

Table 2. Summary of the effects of high concentrations of hCGβ and hCGβcf on the measured hCG concentration as determined by 9 quantitative hCG assays.[a]

Assay	Assay measures hCGβ	Effect of hCGβ on measurement of intact hCG[a]	Assay measures hCGβcf	Effect of hCGβcf on measurement of intact hCG[a]
Advia Centaur Total hCG	Yes[b]	Falsely decreased at ≥970 000 pmol/L	No[b]	No effect
AIA-1800 ST Total β-hCG	Yes[b]	No effect	No[b]	Falsely decreased at ≥250 000 pmol/L
Architect Total β-hCG	Yes[b]	No effect	No[b]	Falsely decreased at ≥1 000 000 pmol/L
Cobas e411 hCG Stat	No[b]	No effect	No[b]	No effect
Dimension RxL hCG	No[b]	No effect	No[b]	No effect
Dxl Total βhCG	Yes[b]	No effect	No[b]	Falsely decreased at ≥3750 pmol/L
Modular Analytics e170 hCG+β	Yes[b]	No effect	Yes	No effect[b]
Immulite 2000 hCG	Yes[b]	No effect	Yes[b]	No effect
Vitros ECi Total β-hCG II	Yes[c]	Falsely decreased at ≥240 000 pmol/L	No[c]	No effect

[a] Falsely decreased results were defined as those in which the measured hCG concentration was ≤50% of expected.
[b] Whittington et al. *(12)* and Sturgeon et al. *(13)*.
[c] Whittington et al. *(12)*.

Grenache et al., 2010

Free β-Subunit of Human Chorionic Gonadotropin in Serum Is a Diagnostically Sensitive Marker of Seminomatous Testicular Cancer

Anna Lempiäinen,[1*] Ulf-Håkan Stenman,[1] Carl Blomqvist,[2] and Kristina Hotakainen[1]

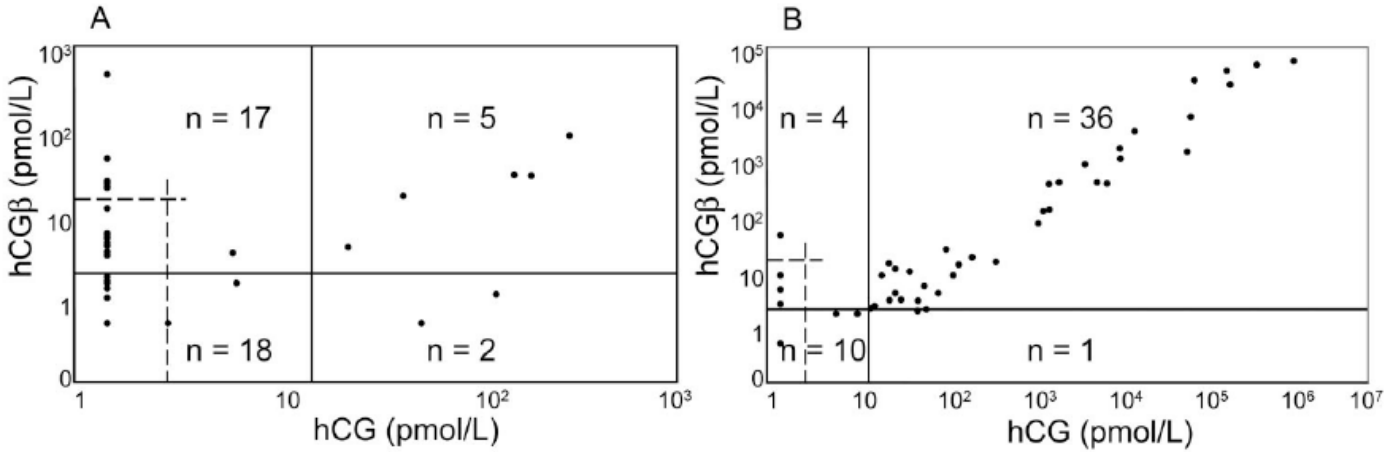


Fig. 1. Preoperative serum concentrations of hCGβ (*y axis*) and hCG (*x axis*) in patients with seminomatous (A) and nonseminomatous (B) testicular cancer.

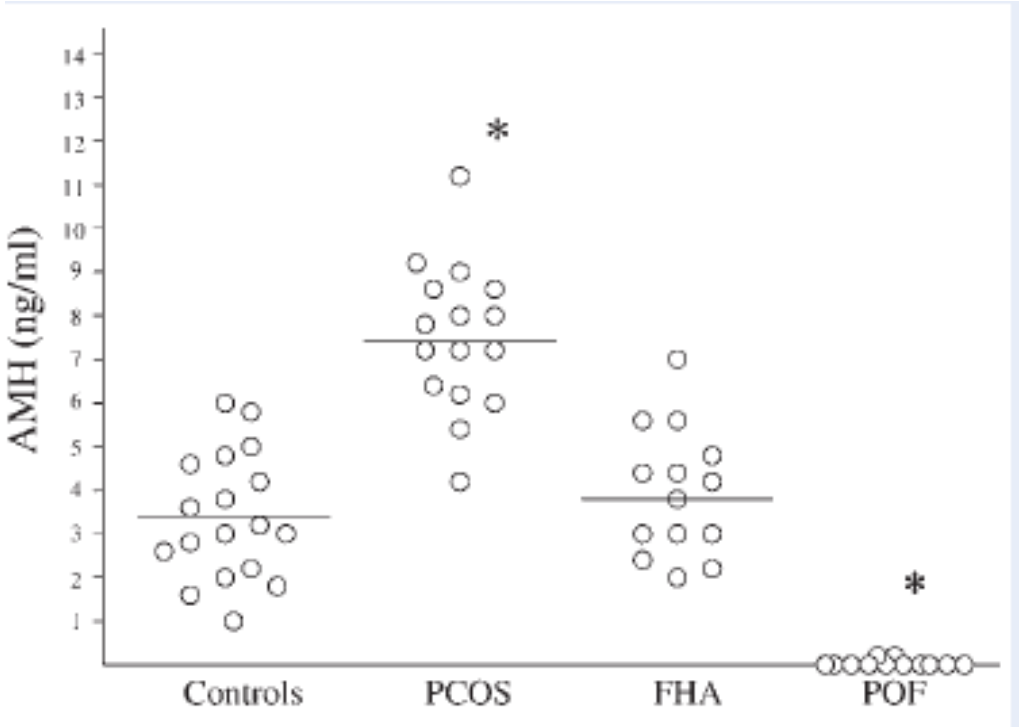
The solid lines mark the upper reference limits and the short dashed lines indicate a concentration of 3 pmol/L (1 IU/L) on the *x axis* and the upper reference limit for the hCG + hCGβ assay (15 pmol/L or 5 IU/L) on the *y axis*.

hCG β is a diagnostically sensitive marker for testicular cancer.

In patients with seminomatous testicular cancer, hCG β is superior to hCG, and in some NSGCT patients it provides additional information.

15-20% of couples complaint of infertility !

Hypogonadotropic amenorrhea



AMH serum levels have been found to be normal in women with hypogonadotropic amenorrhea indicating that initial follicle recruitment is not abolished in hypogonadotropic hypogonadism

La Marca et al., 2009

A Longitudinal Marker for Ovarian Reserve

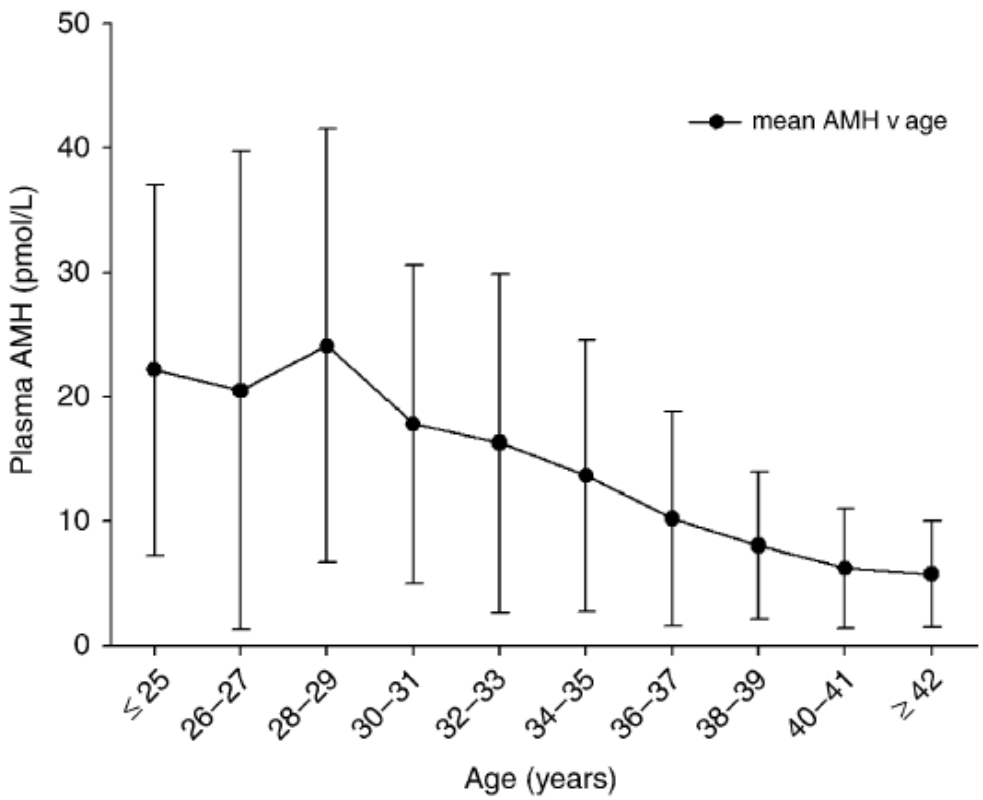
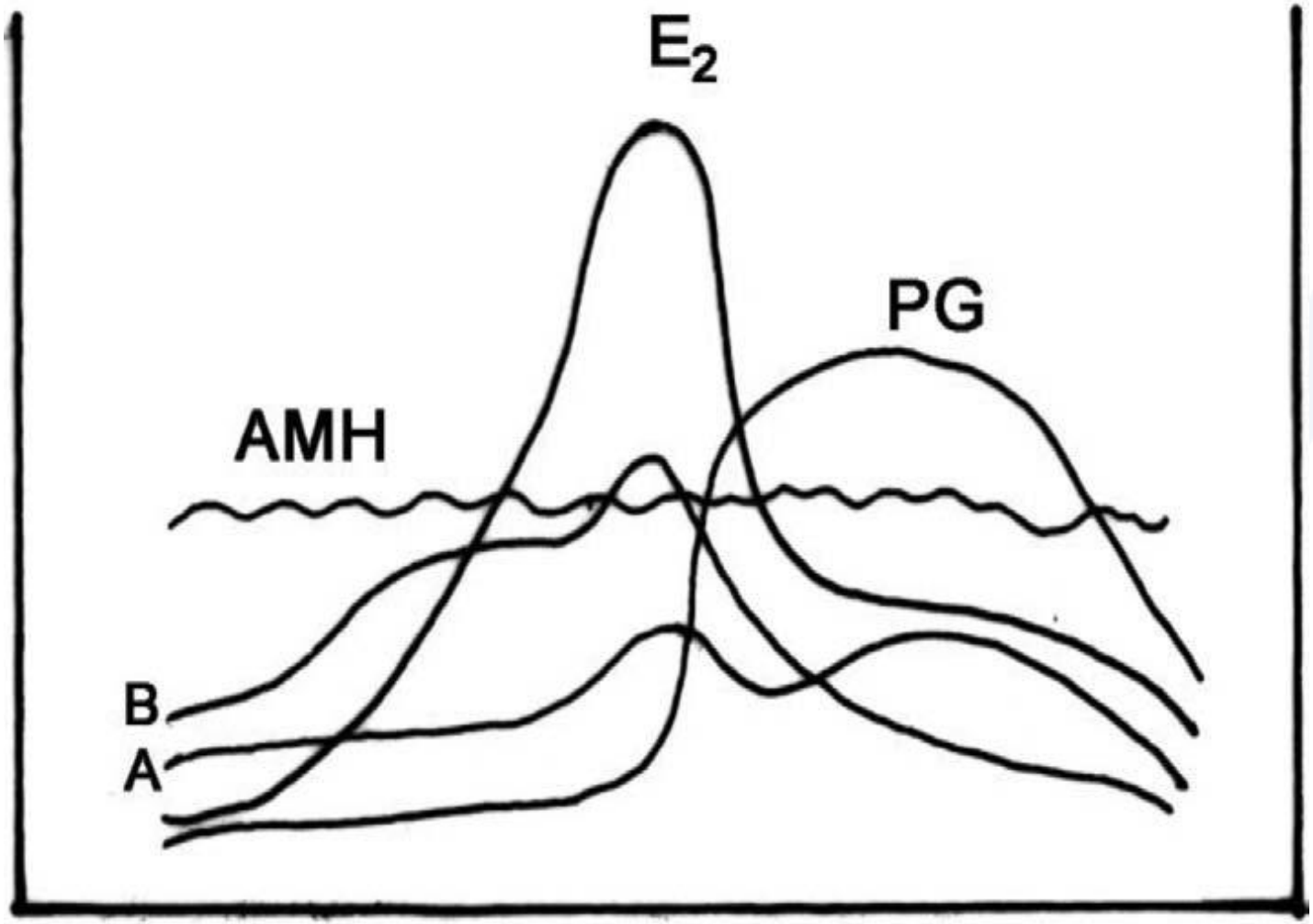


Figure 1 Early follicular phase (day 3–5) anti-müllerian hormone (AMH) over the reproductive age range. Mean ± standard deviation plotted.

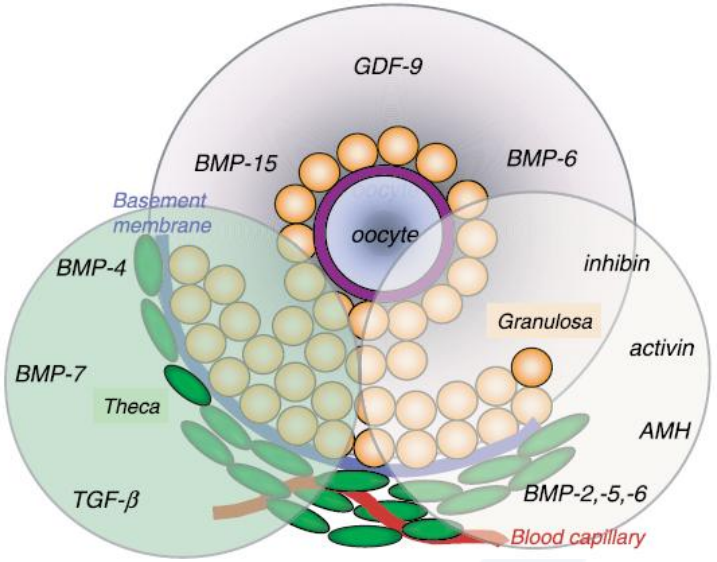
Tremellen et al., 2005

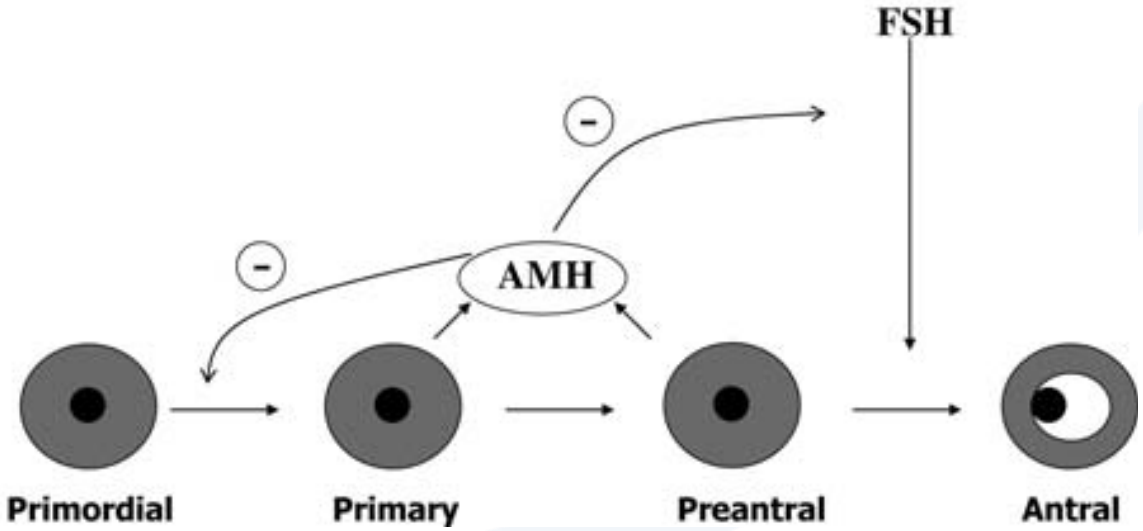
A Longitudinal Marker for Ovarian Reserve



Actions of AMH in the ovary

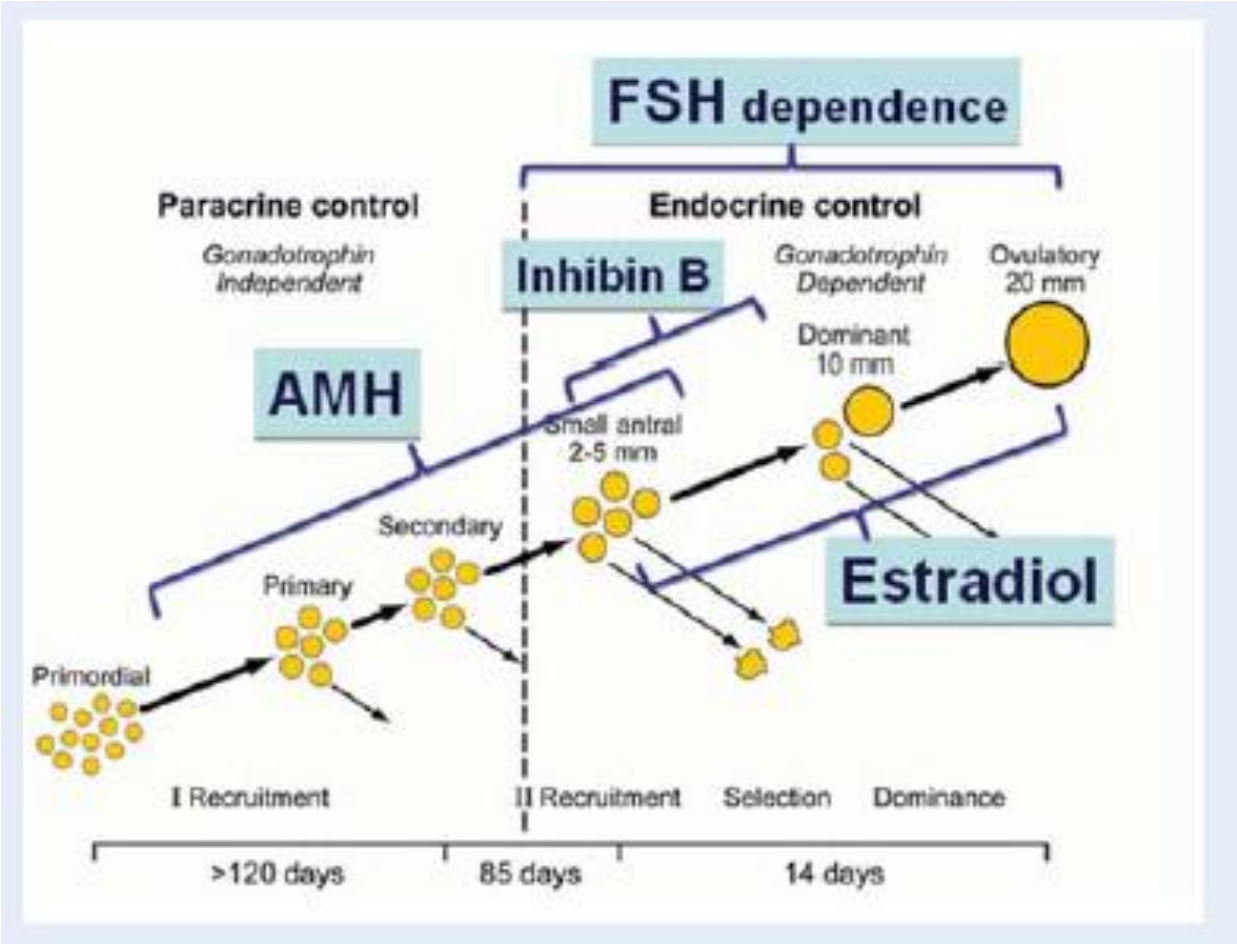
- ✓ Inhibition of follicular activation and growth
- ✓ Inhibition of FSH stimulated growth
- ✓ Inhibition of GC growth
- ✓ Inhibition of aromatase

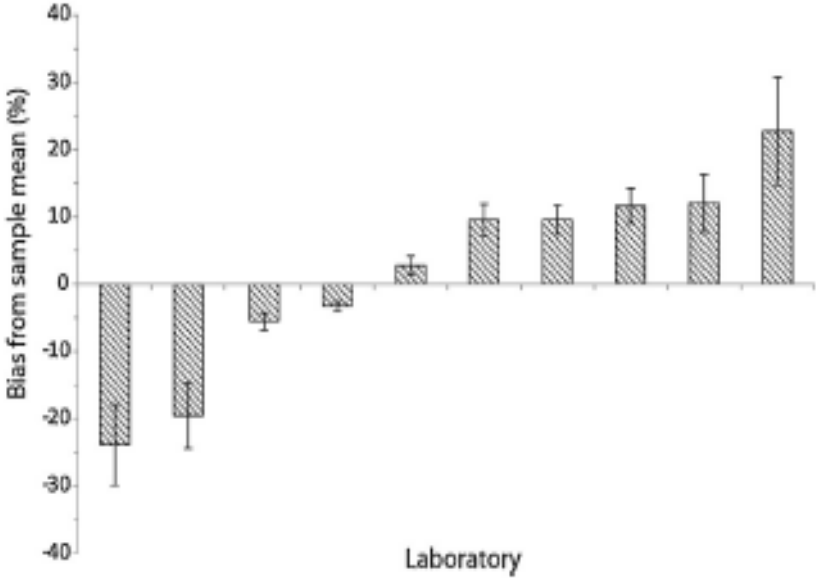


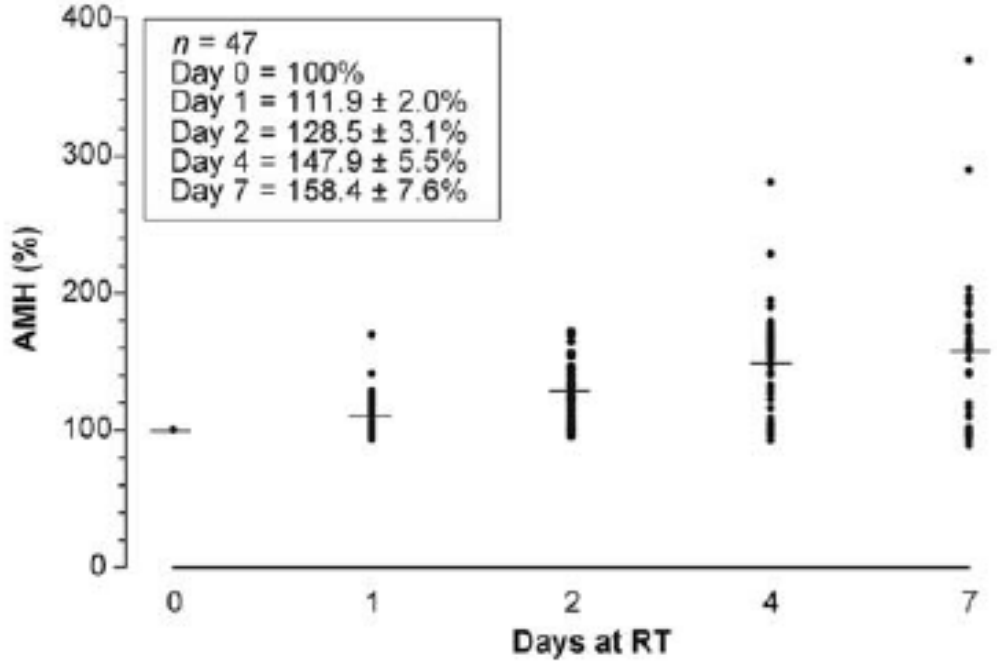


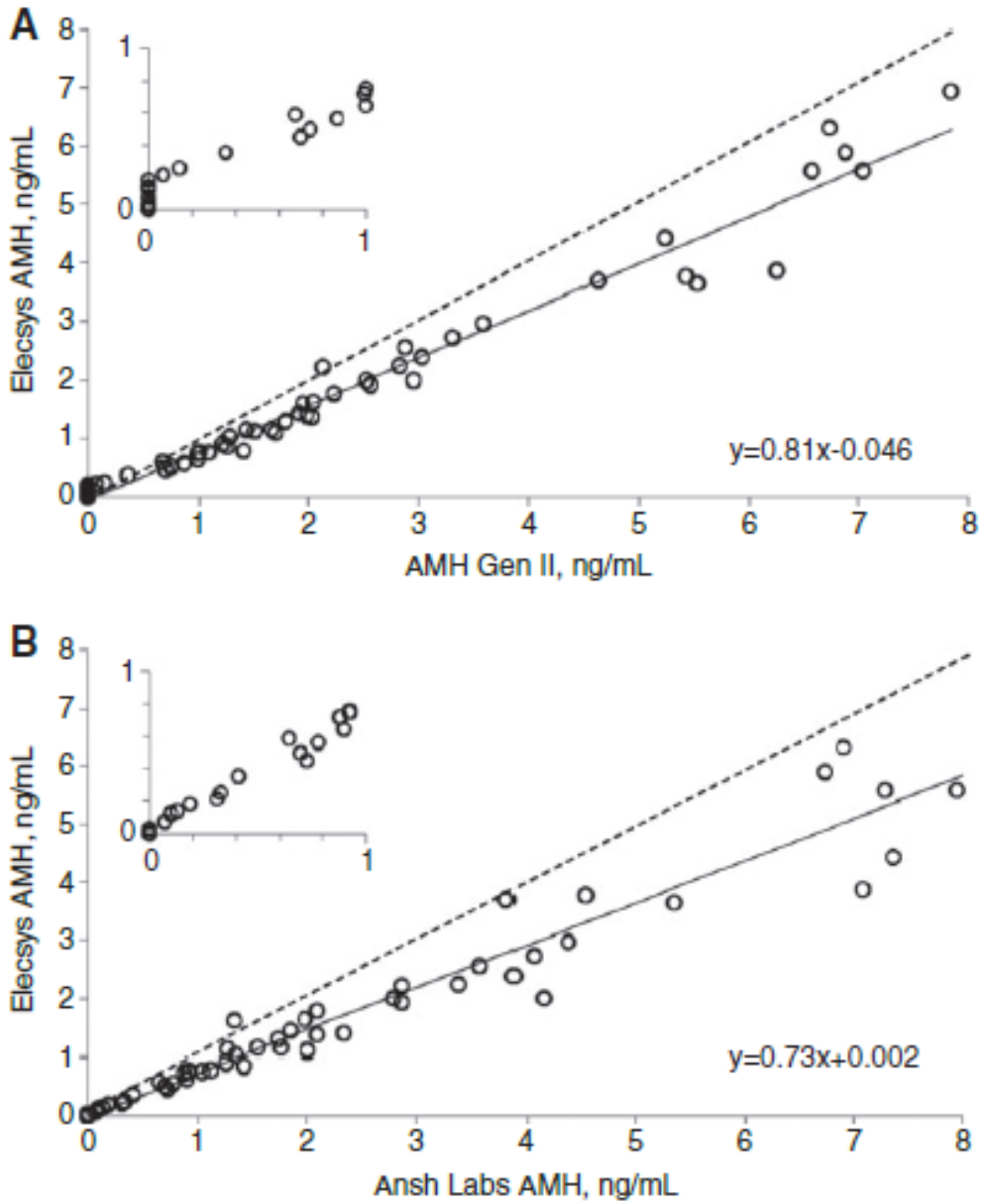
La Marca et al., 2009

A Longitudinal Marker for Ovarian Reserve




Bias from sample mean (%)
40
30
20
10
0
-10
-20
-30
-40
Laboratory


n = 47
Day 0 = 100%
Day 1 = 111.9 ± 2.0%
Day 2 = 128.5 ± 3.1%
Day 4 = 147.9 ± 5.5%
Day 7 = 158.4 ± 7.6%
AMH (%)
400
300
200
100
0
0
1
2
4
7
Days at RT



Gassner et al., 2014

Field Safety Notice regarding the AMH Gen II ELISA.

Summary answer
We demonstrated that C1q in the sample binds to the immobilized anti-AMH antibody forming a complex, initiating the complement cascade. Subsequent C3 activation prevents AMH in the sample from binding to the capture antibody resulting in falsely lowered values. A pre-mix step resolves apparent perceived sample instability and dilution issues

What is known already
Studies suggest sample storage conditions affect AMH Gen II assay results: undiluted fresh samples or samples stored at -20 ° C for < one year or at – 70 ° C result in falsely lowered AMH values.
Calibrators, internal Beckman controls and the UK NEQAS AMH scheme using processed/stored samples do not demonstrate the issue.
Complement is a recognized source of interference in some immunoassays causing a confusing pattern of interference easily mistaken for analyte instability in serum.

Study design, size, duration
Studies to demonstrate the mechanism of complement interference included using fresh/frozen samples with native AMH and also samples in complement-negative synthetic matrix spiked with C1q or AMH.

Participants/materials, setting, methods
The samples were tested in a modified AMH Gen II assay in which the AMH Gen II conjugate was substituted with anti-C1q or anti-C3 antibodies. A pre-mix step was introduced to eliminate complement interference. Its validity was investigated in dilution recovery experiments and by performing fresh sample comparisons versus both the original testing protocol and the alternative AMH assay with the pre-mix step.

100% Chti ?

100% Chti ?

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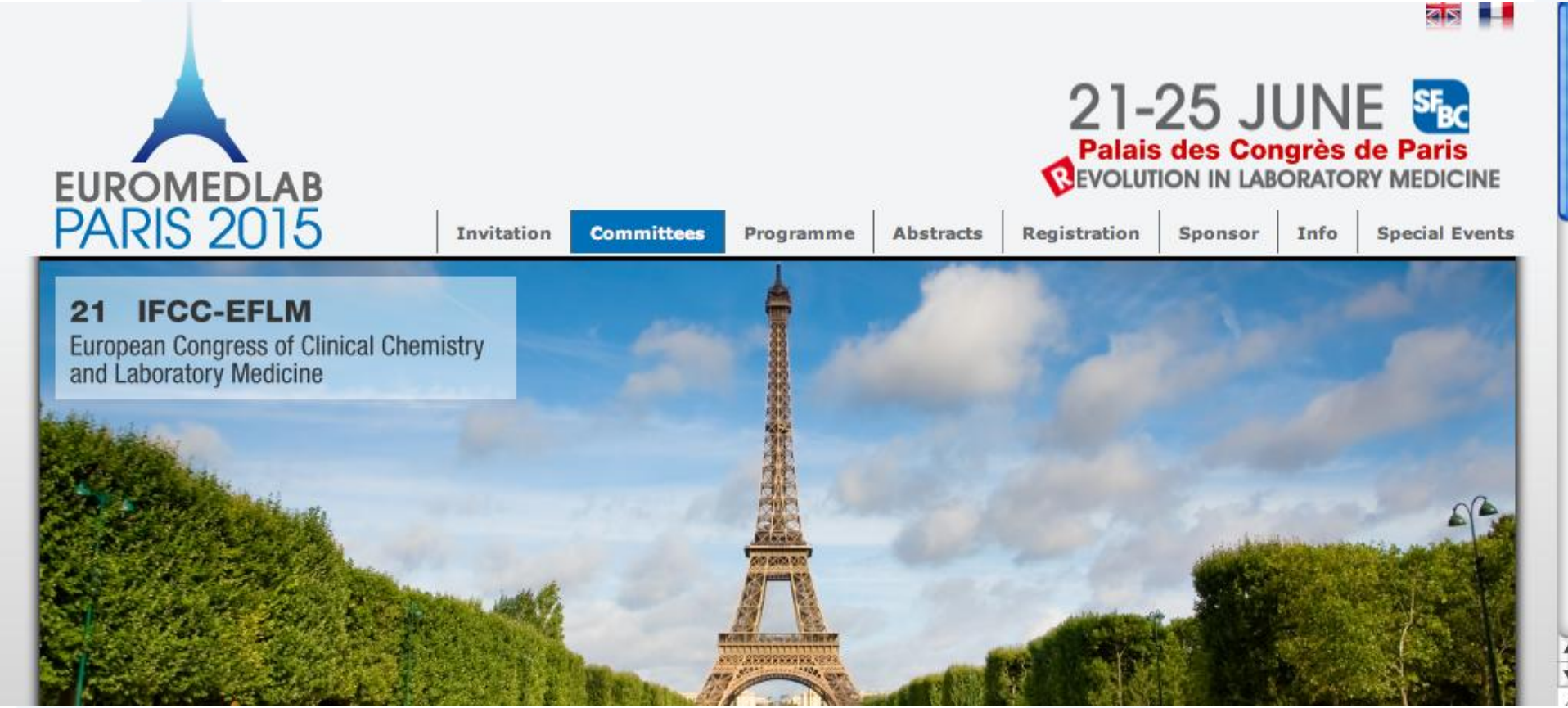

De très bonnes fêtes de fin d'année !

Thank you very much….

…for your attention !

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