IL LABORATORIO E L' IPERANDROGENISMO

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Conflitti di interesse

Ai sensi dell'art. 3.3 sul conflitto di interessi, pag 17 del Regolamento Applicativo Stato-Regioni del 5/11/2009, dichiaro che negli ultimi 2 anni ho avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario:

IPERANDROGENISMO

Sindrome clinicamente eterogenea determinata da un eccesso di androgeni circolanti o da una ipersensibilità tessutale agli androgeni

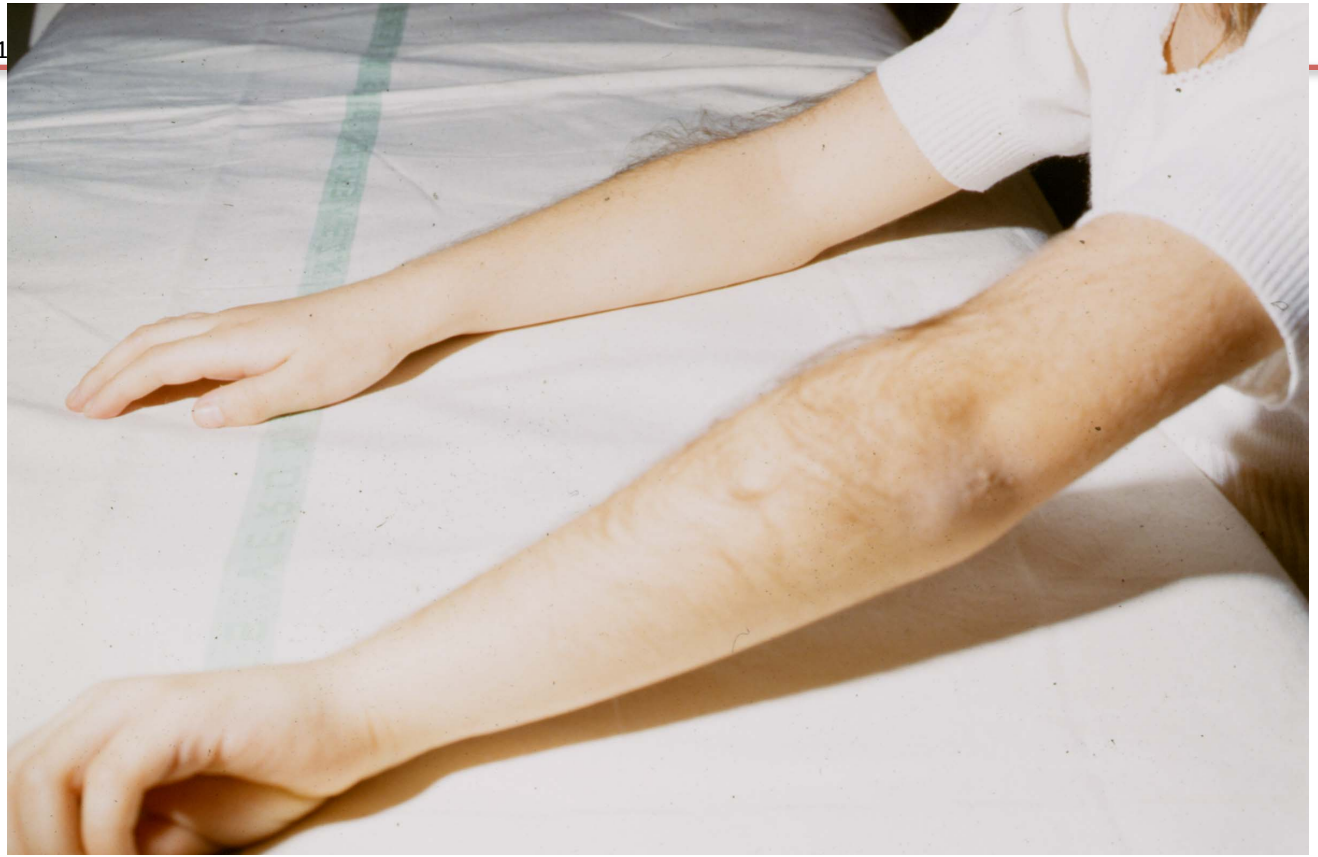
DEFINIZIONI

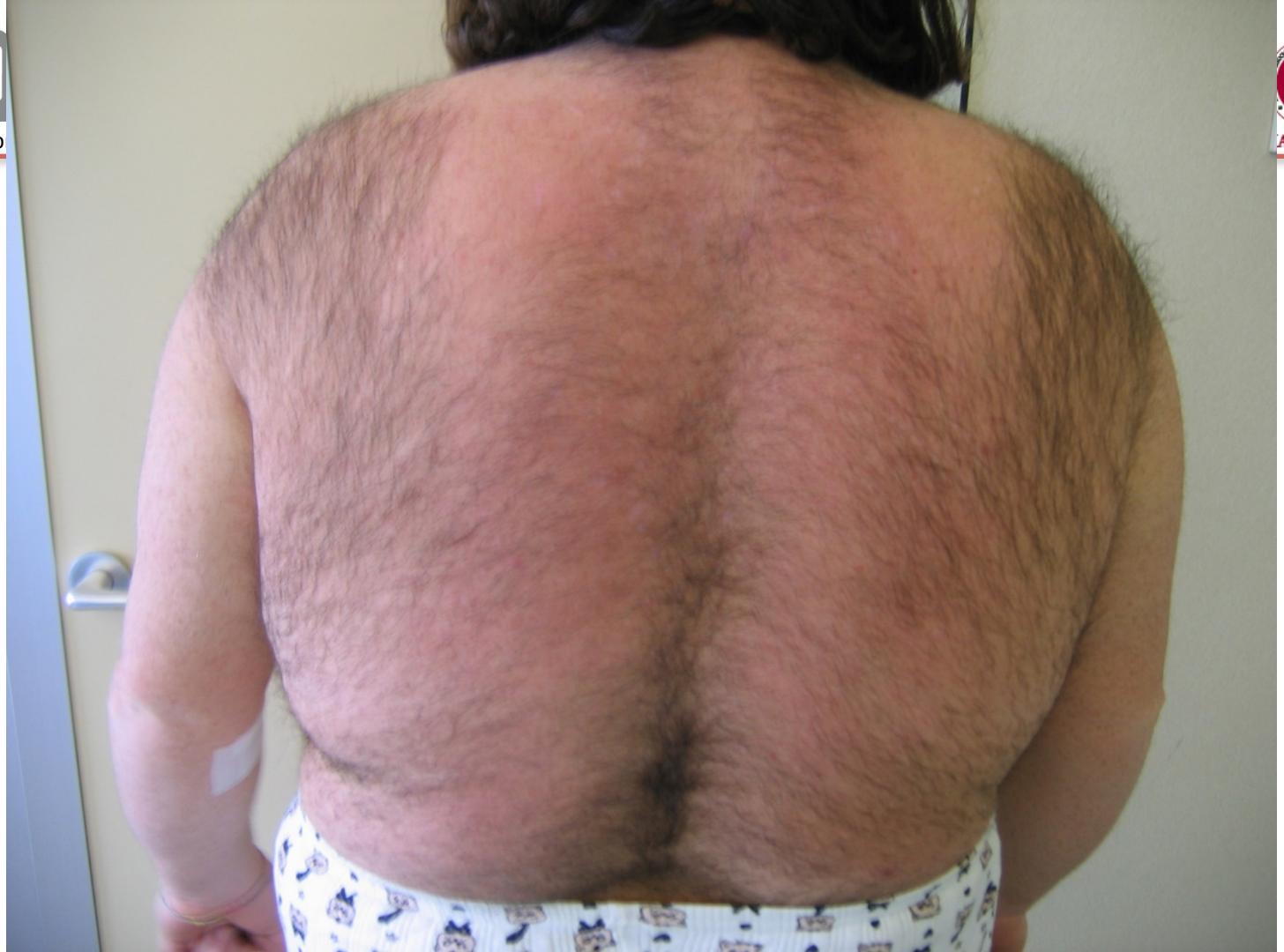
Irsutismo:

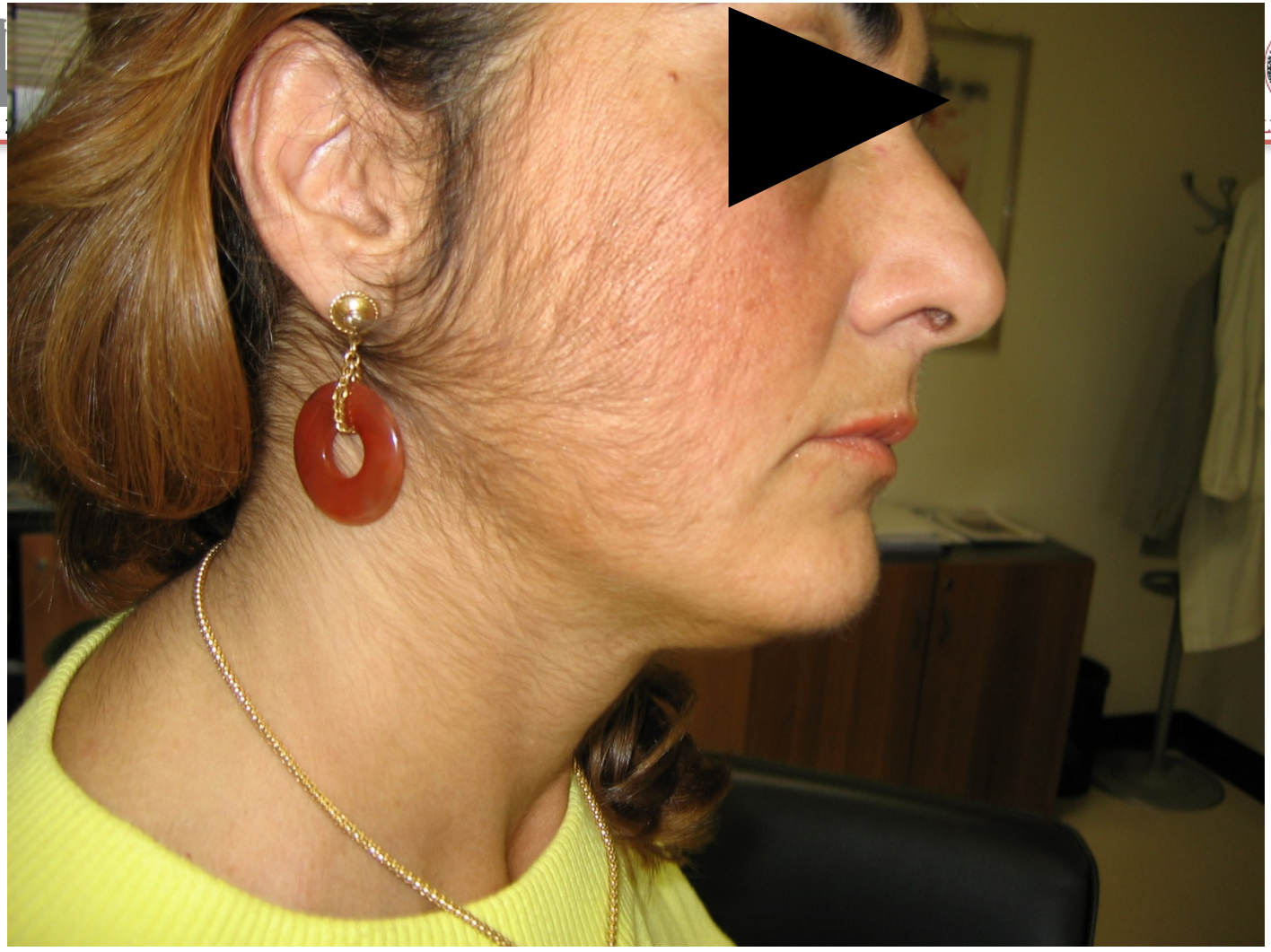
Eccessiva crescita di peli terminali in sedi caratteristiche del sesso maschile

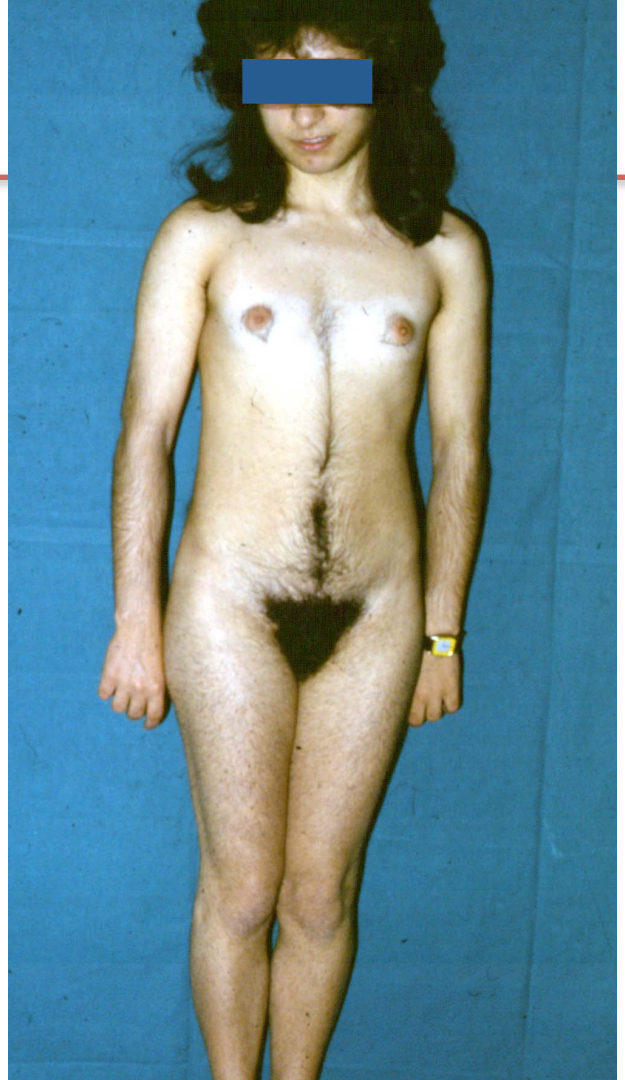
Ipertricosi:

Eccessiva crescita di peli in sedi e con caratteristiche normali per la donna



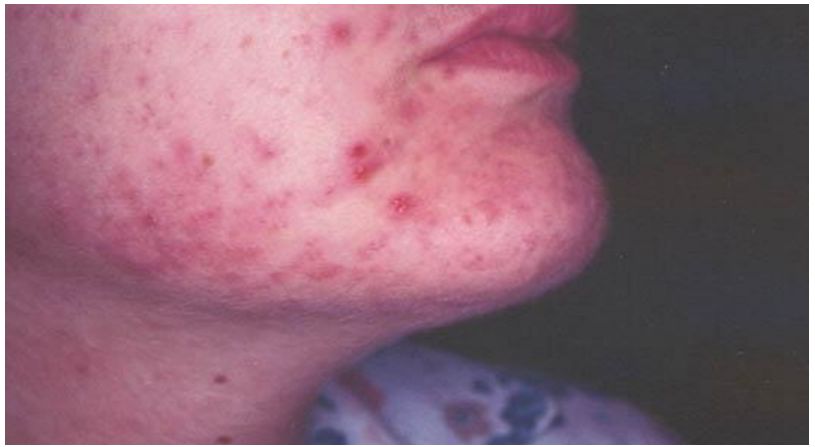




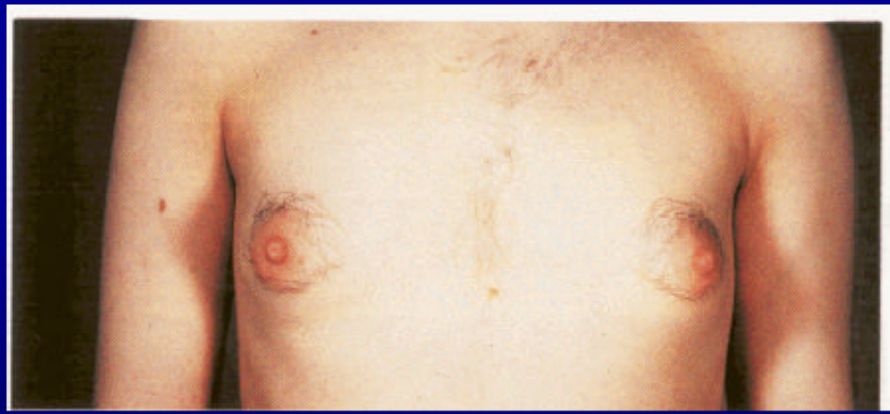


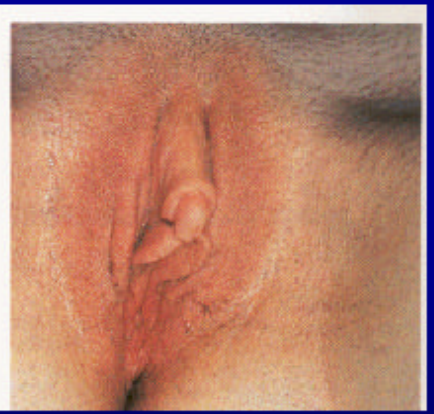
Altre Manifestazioni cliniche

- ✓ Acne
- ✓ Alopecia
- ✓ Acanthosis



VIRILISMO





MICROMASTIA

IPERTROFIA CLITORIDEA

Cause di iperandrogenismo

EXTENSIVE CLINICAL EXPERIENCE

Relative Prevalence of Different Androgen Excess Disorders in 950 Women Referred because of Clinical Hyperandrogenism

E. Carmina, F. Rosato, A. Jannì, M. Rizzo, and R. A. Longo

The Journal of Clinical Endocrinology & Metabolism 91(1):2–6

doi: 10.1210/jc.2005-1457

	N° di pazienti	% sul totale delle pazienti
Sindrome dell'Ovaio Policistico	685	72.1
Iperandrogenismo Idiopatico	150	15.8
Irsutismo Idiopatico	72	7.6
NCAH	41	4.3
Neoplasie Androgeno-Secernenti	2	0.2

Quali esami chiedereste per un inquadramento diagnostico in una donna irsuta con ciclo regolare?

1) 17OH-progesterone + Testosterone totale

2) 1 + Androstenedione e DHEA-S

3) 1 + Testosterone libero

4) nessun esame

N ENGL J MED 353;24 WWW.NEJM.ORG DECEMBER 15, 2005

The NEW ENGLAND JOURNAL of MEDICINE

CLINICAL PRACTICE

Hirsutism

Robert L. Rosenfield, M.D.

If hirsutism is mild (i.e., with a Ferriman–Gallwey score of 8 to 15) and menses are regular, with none of the features described above to suggest a secondary cause, it is reasonable to forgo laboratory evaluation, given the very high likelihood that the hirsutism is idiopathic. (Historically, hirsutism

SPECIAL FEATURE

Clinical Practice Guideline

Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society Clinical Practice Guideline

Kathryn A. Martin, R. Jeffrey Chang, David A. Ehrmann, Lourdes Ibanez, Rogerio A. Lobo, Robert L. Rosenfield, Jerry Shapiro, Victor M. Montori, and Brian A. Swiglo

1.1 Diagnosis of hirsutism

1.1.1 We suggest against testing for elevated androgen levels in women with isolated mild hirsutism because the likelihood of identifying a medical disorder that would change management or outcome is low (2⊕○○○).

JCEM 2008; 93: 1105-1120

Neoplasie androgeno-secernenti

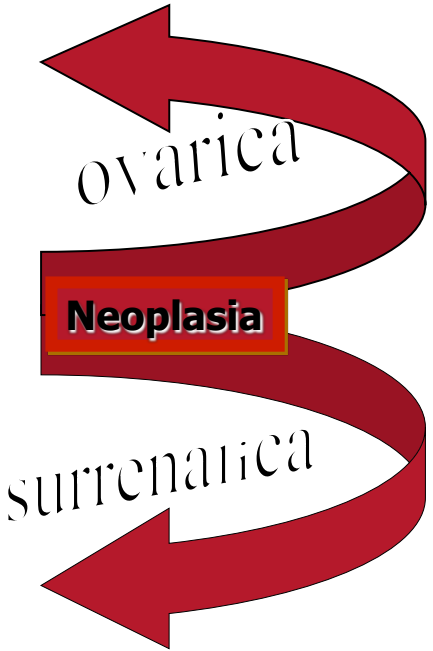
- **Insorgenza improvvisa**
- **Grado di irsutismo elevato**
- **Rapida evoluzione dell'irsutismo**

Testosterone > 2 ng/ml

DHEAS < 3 µg/ml

Testosterone > 2 ng/ml

DHEAS > 6 µg/ml



Iperandrogenismo biochimico

- C'è mancanza di chiarezza su:
  1. quale androgeno misurare,
  2. con che frequenza,
  3. quali sono i valori normali
  4. che tecnica analitica sia preferibile impiegare.

Clin Endocrinol. 2007;67(6):811-815

DETERMINAZIONE degli androgeni

- Quale testosterone ? Con che metodo?
- Quali altri androgeni ?

} Laboratorio

Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society Clinical Practice Guideline FREE

Kathryn A Martin ✉, R Rox Anderson, R Jeffrey Chang, David A Ehrmann, Rogerio A Lobo, M Hassan Murad, Michel M Pugeat, Robert L Rosenfield

Author Notes

The Journal of Clinical Endocrinology & Metabolism, Volume 103, Issue 4, April 2018, Pages 1233–1257, https://doi.org/10.1210/jc.2018-00241

Published: 07 March 2018 **Article history** ▾

1.0 Diagnosis of hirsutism

1.1. We suggest testing for elevated androgen levels in all women with an abnormal hirsutism score (2 |⊕⊕OO). In those cases where serum total testosterone levels are normal, if sexual hair growth is moderate/severe or sexual hair growth is mild but there is clinical evidence of a hyperandrogenic endocrine disorder (such as menstrual disturbance or progression in spite of therapy), we suggest measuring an early morning serum total and free testosterone by a reliable specialty assay. (2 |⊕⊕OO)

TESTOSTERONE: laboratorio

Pre-analitiche

PRELIEVO - a digiuno nelle prime ore del mattino e nei primi 10 giorni del ciclo

Analitiche

METODI – sensibilità, specificità, esattezza, ripetibilità, equilibrio forma libera e legata

STANDARDIZZAZIONE e ARMONIZZAZIONE

Post-analitiche

REFERTO – Intervalli di riferimento per metodo, per sesso ed età

J Clin Endocrinol Metab 2007;92:405-13

TESTOSTERONE: metodi

Tecniche immunometriche

- RIA dopo estrazione cromatografica che permetteva la concentrazione del campione
- Dirette RIA, ELISA, CLIA, ECLIA con tecniche di amplificazione del segnale

⇒semplici, rapidi, poco costosi, automatizzabili con produttività elevata,

⇒imprecisi a concentrazioni basse, poco standardizzati, poco specifici per interferenze con altri steroidi, precursori o metaboliti

TESTOSTERONE: metodi

Cromatografia Liquida con la Spettrometria di Massa tandem (LC-MS/MS) consiste in estrazione, separazione cromatografica, vaporizzazione e ionizzazione degli analiti, frammentazione e rilevazione dei diversi analiti separati

⇒Nella stessa corsa cromatografica possono essere misurati più steroidi, può dare misure accurate e sensibili

⇒Costosa, produttività limitata, poco standardizzata, necessità di personale specializzato, diverse fasi delicate per l'introduzione di errori, attrezzature idonee, IR poco collaudati.

TESTOSTERONE: metodi

Clinical Chemistry 61:12
1475–1483 (2015)

Endocrinology and Metabolism

Comparison of 7 Published LC-MS/MS Methods for the Simultaneous Measurement of Testosterone, Androstenedione, and Dehydroepiandrosterone in Serum

Rahel M. Büttler,[1] Frans Martens,[1] Flaminia Fanelli,[2] Hai T. Pham,[3] Mark M. Kushnir,[4,5] Marcel J.W. Janssen,[6] Laura Owen,[7] Angela E. Taylor,[8] Tue Soeborg,[9] Marinus A. Blankenstein,[1] and Annemieke C. Heijboer[1*]

BACKGROUND: Recently, LC-MS/MS was stated to be the method of choice to measure sex steroids. Because information on the mutual agreement of LC-MS/MS methods is scarce, we compared 7 published LC-MS/MS methods for the simultaneous measurement of testosterone, androstenedione, and dehydroepiandrosterone (DHEA).

METHODS: We used 7 published LC-MS/MS methods to analyze in duplicate 55 random samples from both men and women. We performed Passing–Bablok regression analysis and calculated Pearson correlation coefficients to assess the agreement of the methods investigated with the

CONCLUSIONS: In general, the LC-MS/MS methods investigated show reasonable agreement. However, some of the assays show differences in standardization, and others show high variation.

The measurement of hormone concentrations is vital for clinical endocrinology as well as endocrine research. Since the beginning of the 21st century, increased attention has been paid to the accuracy of hormone measurements, especially in steroid hormone analysis. In 2003, Taieb et al. showed that commonly used immunoassays

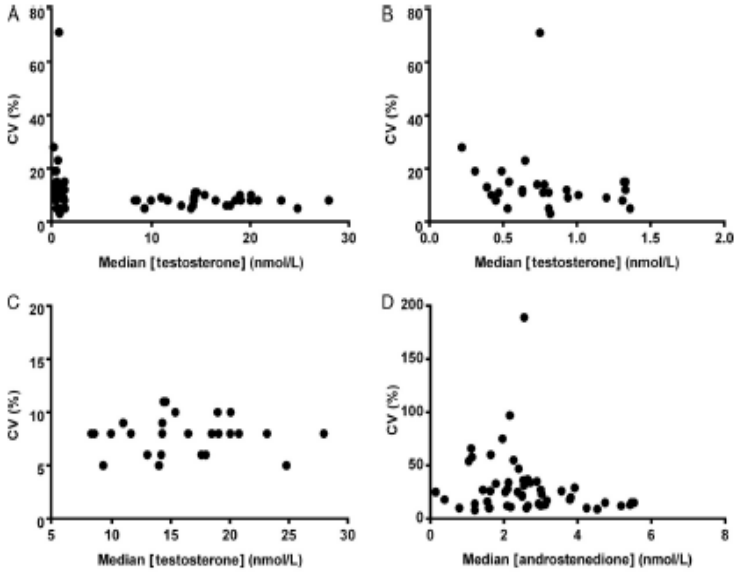


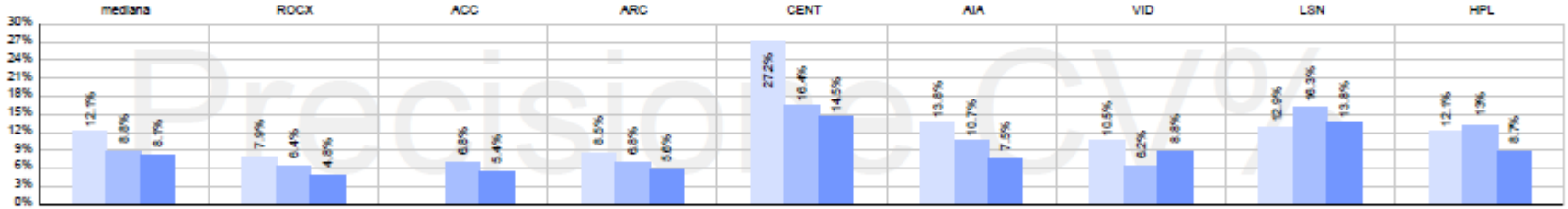
Fig. 5. Intermethod variation.
Intermethod variation was calculated per sample with the formula CV (%) = SD/(median concentration of all methods). CVs per sample for all testosterone values (A), testosterone in women (B), testosterone in men (C), androstenedione (D), androstenedione with exclusion of the values measured by method F (E), and DHEA (F).

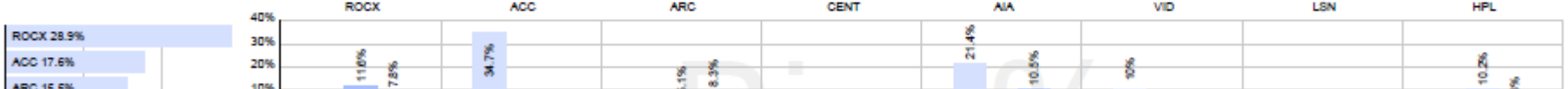
TESTOSTERONE: metodi

Valutazione delle prestazioni dei metodi

Testosterone **microg/L** *Riepilogo cumulativo dal campione* ***IM71a*** *al campione* ***IM76c*** *(ciclo 2017)*

	CONS 7389 (100%)		ROCX 2132 (28.9%)		ACC 1297 (17.6%)		ARC 1142 (15.5%)		CENT 862 (11.7%)		AIA 516 (7%)		VID 511 (6.9%)		LSN 166 (2.3%)		HPL 108 (1.5%)	
IM73c (P160)	0.49	23.6%	0.45	8.7%	0.64	7.7%	0.41	8.1%	0.42	32.8%	0.59	12.1%	0.55	11.7%	0.21	17.3%	0.37	12%
IM75b (P160)	0.5	25.7%	0.46	7.1%	0.69	8.1%	0.4	8.8%	0.39	21.3%	0.61	8.3%	0.54	11%	0.22	10.4%	0.36	12.1%
IM71a (P156)	0.75	21.9%	0.89	7.6%	0.71	7.3%	0.81	6.4%	0.7	25.9%	0.55	15.9%	0.53	8.5%	0.64	11.6%	0.85	17.1%
IM75a (P156)	0.78	19.1%	0.89	5.5%	0.72	7.8%	0.83	7%	0.84	14.8%	0.57	11.7%	0.52	10.8%	0.68	14.3%	0.85	6.7%
IM71b (P163)	1.12	15.8%	1.16	7%	1.09	7.6%	1.1	9.4%	1.15	14.2%	1.15	15.4%	0.86	5%	0.95	7.9%	-	-
IM71c (P161)	1.94	11.2%	1.98	5.7%	1.92	4.6%	1.97	6.6%	1.76	8.6%	2.85	10.1%	1.6	6%	1.27	19.8%	2.1	17%
IM74b (P161)	2.01	12.2%	2.03	4.6%	2	5.6%	2.07	5.9%	1.82	7%	2.58	7.6%	1.6	6.7%	1.27	18.2%	2.16	1.7%
IM73b (P162)	2.02	23.2%	2.51	8.6%	1.73	5.1%	2.41	7%	2.08	33.5%	1.87	11.3%	1.6	8.8%	1.6	20.4%	-	-
IM72a (P157)	2.23	16.7%	2.57	6.1%	1.95	5.6%	2.43	5.5%	2.07	21.4%	2.01	8.8%	1.71	5.5%	1.83	10.5%	2.49	13.8%
IM74a (P157)	2.25	15.4%	2.58	4.8%	1.94	5.9%	2.45	5.7%	2.32	15%	1.94	8.4%	1.77	7.8%	1.63	23.4%	2.2	6.2%
IM74c (P155)	2.29	14.2%	2.44	4.1%	2.06	5.8%	2.49	5.4%	2.03	11.4%	2.78	6.9%	1.82	6%	1.51	16.2%	2.34	3.2%
IM75c (P155)	2.3	14.2%	2.44	4%	2.05	5.8%	2.49	5.7%	2.02	10.6%	2.78	6.9%	1.82	6%	1.51	16.2%	2.34	3.2%
IM76c (P155)	2.3	14.1%	2.43	4.1%	2.04	5.2%	2.49	5.5%	2.02	10.7%	2.78	6.9%	1.82	6%	1.51	16.2%	2.34	3.2%
IM72b (P164)	3.65	19.8%	4.03	7.2%	3.36	7.7%	3.73	6.1%	3.22	16.5%	4.34	6.4%	2.97	5.1%	2.8	14.7%	-	-
IM72c (P159)	5.13	13.8%	5.32	5.8%	4.45	5.1%	5.73	6.2%	4.59	15.3%	6.23	7.3%	4.39	6.2%	3.48	6.2%	5.09	6.3%
IM76b (P159)	5.22	12.5%	5.43	4.6%	4.51	5.2%	5.68	4.8%	5.19	11%	6.2	6.4%	4.49	11.2%	3.69	18.5%	5.12	9.5%
IM73a (P158)	7.16	13.2%	7.85	4.5%	5.83	4.6%	7.77	6.2%	6.41	10.8%	7.62	7.1%	7.38	9.7%	6.27	11.8%	6.83	6.3%
IM76a (P158)	7.21	13.1%	7.85	4.3%	5.79	5%	7.73	5.2%	6.61	10.3%	7.72	7.9%	7.67	8.3%	6.55	12.3%	6.83	6.6%





TESTOSTERONE: *CRITICITA' POST-ANALITICHE*

DE GRUYTER Clin Chem Lab Med 2018; aop

Giacomo Montagna, Samuela Balestra, Federica D'Aurizio, Francesco Romanelli, Cinzia Benagli, Renato Tozzoli, Lorenz Risch, Luca Giovanella* and Mauro Imperiali

Establishing normal values of total testosterone in adult healthy men by the use of four immunometric methods and liquid chromatography-mass spectrometry

https://doi.org/10.1515/cclm-2017-1201
Received December 22, 2017; accepted April 23, 2018

LC-MS/MS and the other IMAs. Age-specific T concentrations in non-obese (BMI <29.9 kg/m²) men were greater than in all men. The total T normal range in non-obese

350 maschi adulti sani

Cromatografia liquida - tandem massa spettrometria

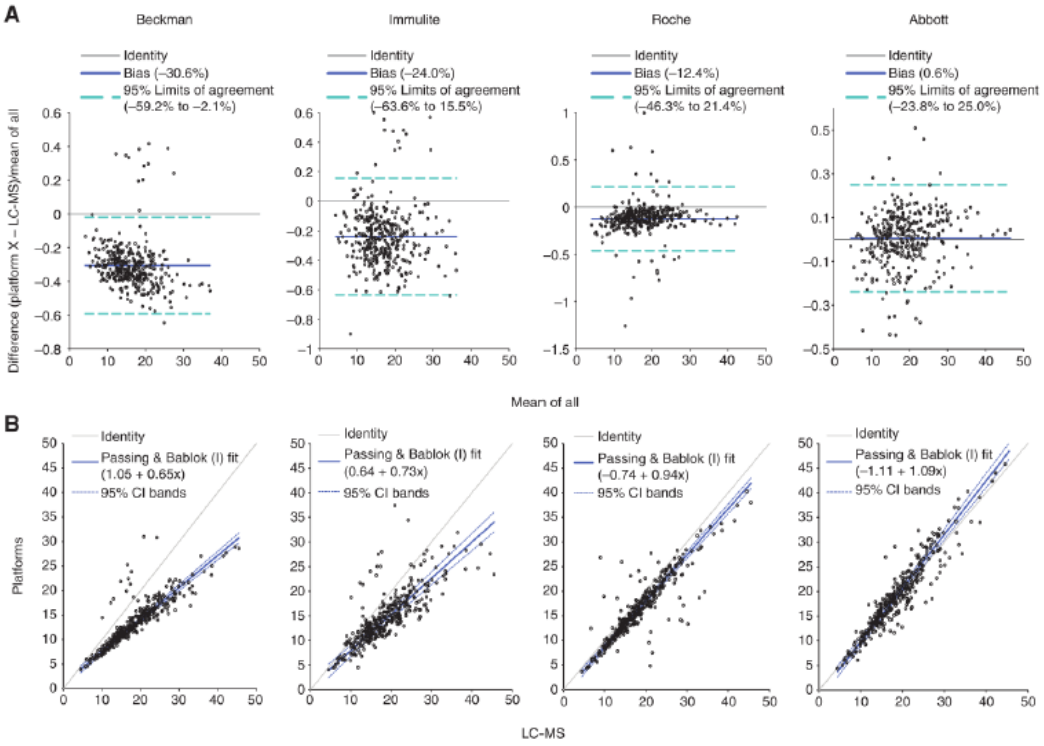


Figure 1: Interassay agreement and differences in serum testosterone levels between four commercial IMAs and LC-MS/MS.

TESTOSTERONE: *CRITICITA' POST-ANALITICHE*

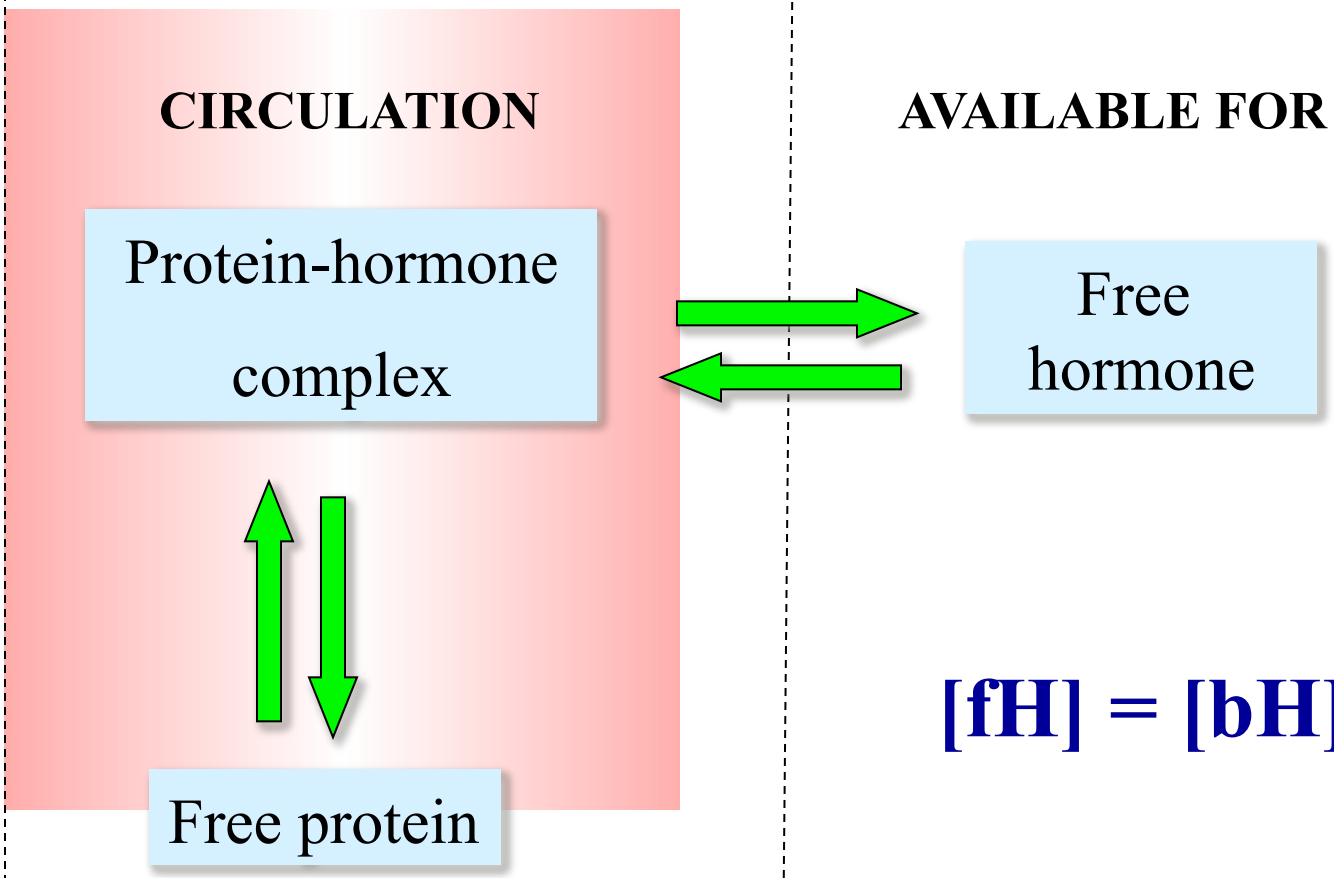
Table 1: Total testosterone distribution (nmol/L) in a cohort of European healthy men measured by five analytical methods.

Total T, nmol/L	n	Min	1st Quartile	Median	95% CI	3rd Quartile	Max	IQR
Beckman	349	3.91	10.53	12.92	12.30–13.80	16.27	30.93	5.75
Immulite	347	4.06	11.12	13.90	12.30–14.60	17.28	37.40	6.17
Roche	345	3.7	12.50	15.70	15.10–18.80	20.13	40.20	7.63
Abbott	345	4.34	14.02	18.04	17.29–18.60	22.87	47.55	8.83
LC-MS	345	4.48	14.16	17.91	17.14–18.84	22.36	45.50	8.20

Table 8: Comparison between our RRs with the one provided by the manufacturers.

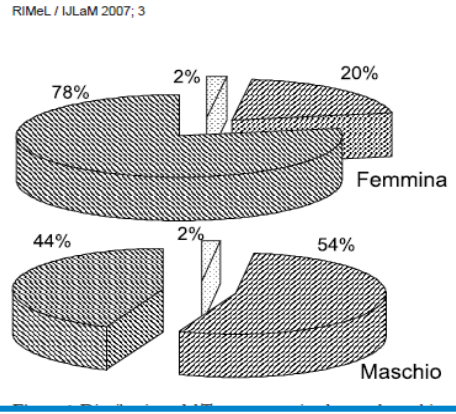
Manufacturer, age range, number of participants	Manufacturer's RRs	Our RRs	χ^2 p-value for URLs	χ^2 p-value for LRLs
Abbott, 21–49, n = 269	1.63–34	7.515–40.41	0.0798	**0.0138**
Abbott, >50, n = 70	4.41–35.38	6.25–39.63	0.5764	0.1543
Immulite, 20–49, n = 269	5.55–25.19	6.01–28.68	**0.0356**	0.5233
Immulite, >50, n = 70	4.47–26.61	5.69–30.35	0.5594	0.3156
LC-MS	8.675–34.7	7.49–34.75	1.0000	0.2678
Beckman, n = 349	6.07–27.1	6.06–25.08	1.0000	1.0000
Roche, 20–49, n = 200	8.65–29	6.01–32.63	0.3998	**0.0192**
Roche, >50, n = 67	6.68–25.7	5.33–32.90	0.3172	0.3100

Testosterone libero

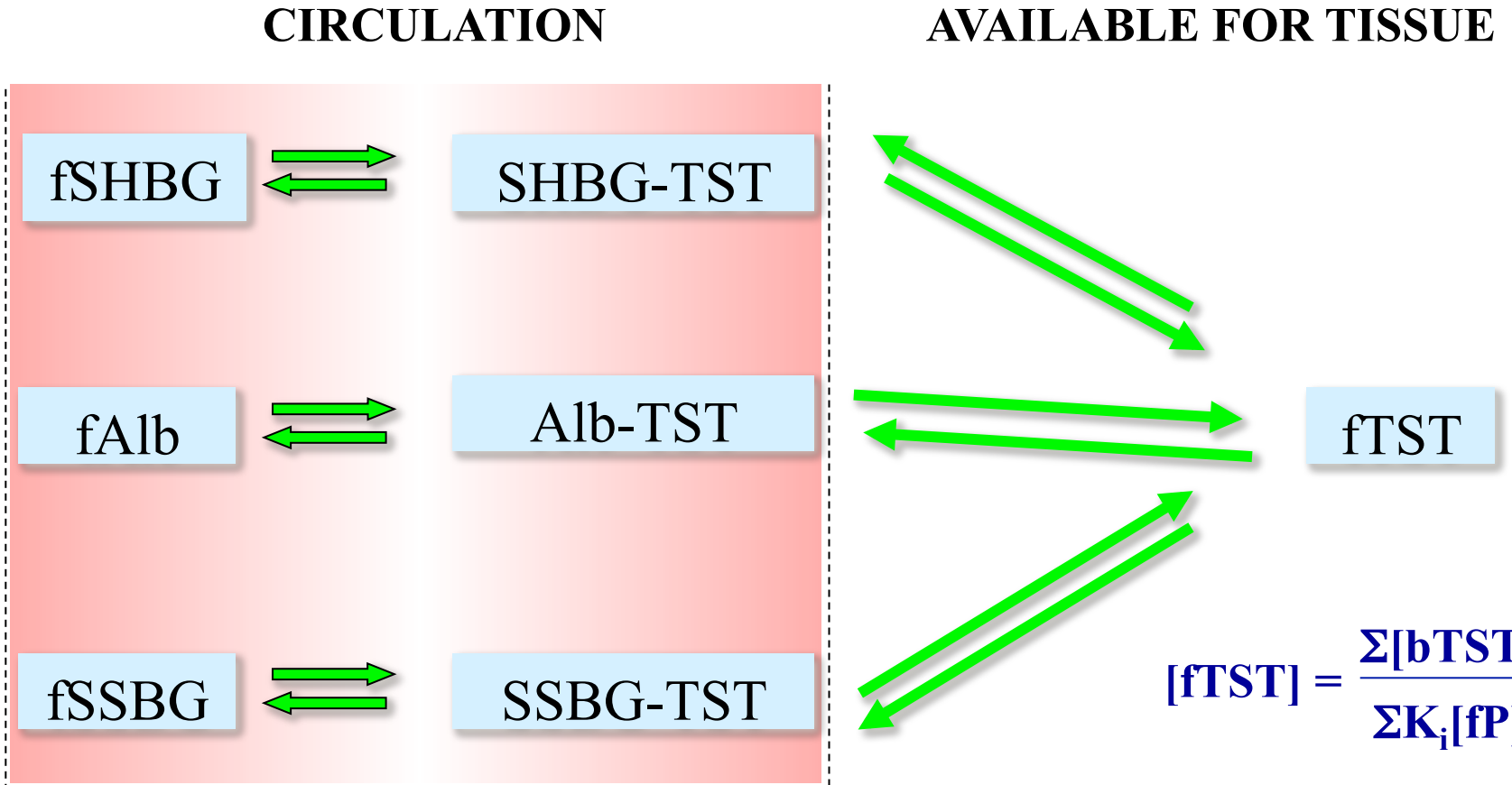


$$[fH] = [bH] / K[fP]$$

Testosterone Totale = Testosterone Libero + T(albumina) + T(SHBG)



TESTOSTERONE libero

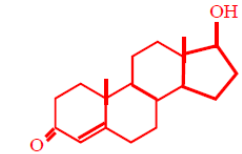


TESTOSTERONE libero

Test immunometrici diretti

I risultati dei Test RIA Diretti mostrano limiti di accuratezza, sensibilità e comparabilità inter-laboratorio

Metodo	*Pregi*	*Difetti*
RIA diretti	1) Semplici, rapidi e relativamente poco costosi 2) Richiedono competenza tecnica minima 3) Automatizzabile	1) Accuratezza, sensibilità e comparabilità inter-laboratori scarsa soprattutto a causa di: - significativo legame dell'analogo alle proteine del siero - [illegible]



Testosterone

Methods for FT assessment
Morales A, et al. Can J Urol 2012;19:6314-8

Equilibrium dialysis (reference method)

Manual, time consuming, costly method

Ultrafiltration (reference method)

Faster and more automated than ED, manual, time consuming, costly method

Tandem mass spectrometry

Time consuming, costly method

Analog FT IMA

'Direct' measurement of FT by RIA and CLIA, commercially available, but considered inaccurate

Calculated FT (FAI)

Ratio of TT and SHBG: unreliable

Calculated FT (cBAT)

cFT, cBAT from TT, SHBG, Albumin: is the preferred method (?)

Calcolo testosterone libero- formula di Vermeulen

sito web www.issam.ch

Free & Bioavailable Testosterone calculator

These calculated parameters more accurately reflect the level of bioactive testosterone than does the sole measurement of total serum testosterone. Testosterone and dihydrotestosterone (DHT) circulate in plasma unbound (free approximately 2 - 3%) ,bound to specific plasma proteins (sex hormone-binding globulin SHBG) and weakly bound to nonspecific proteins such as albumin. The SHBG-bound fraction is biologically inactive because of the high binding affinity of SHBG for testosterone. Free testosterone measures the free fraction, bioavailable testosterone includes free plus weakly bound to albumin.

Albumin [ ] g/dL ▾ Calculate Explanation and examples

SHBG [ ] nmol/L ▾

Testosterone [ ] ng/dL ▾

Free Testosterone [ ]

Bioavailable Testosterone [ ]

Disclaimer: Results from this calculator should NOT be solely relied upon in making (or refraining from making) any decision in any case/ circumstances without the prior consultation of experts or professional persons. No responsability whatsoever is assumed for its correctness or suitability for any given purpose.

WARNING! The calculated free and bioavailable testosterone are reliable in most clinical situations, but should not be relied upon in situations with potential massive interference by steroids binding to SHBG; e.g. in women during pregnancy, in men during treatment inducing high levels of DHT (e.g. transdermal DHT, oral testosterone) or mesterolon

This calculator was developed at the Hormonology department, University Hospital of Ghent, Belgium. If you have suggestions to improve this calculator, or for further questions or help contact us Dr. Tom Fiers or Prof. Dr. J.M. Kaufman

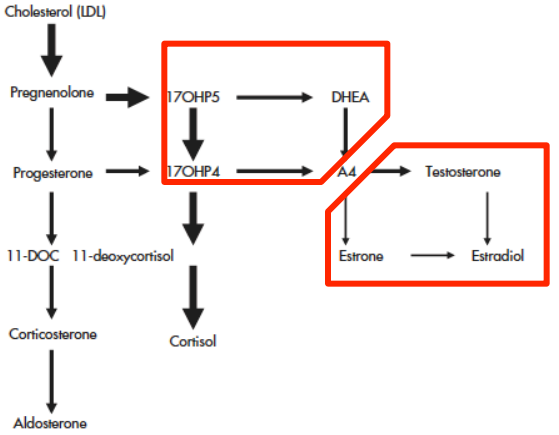
ANDROSTENEDIONE

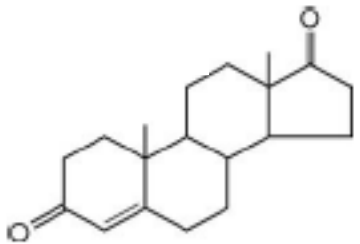
⇒ Aumenti lievi o moderati sono spesso idiopatici

⇒ Solo il 3% di PCO presenta valori aumentati di Δ 4 da solo

⇒ I metodi usati nei laboratori sono 'diretti', poco precisi

ORMONE	ATTIVITA' ANDROGENICA	ORIGINE Surrene	Ovaio	Periferia
Testosterone	100	5-25%	5-25%	50-70%
DHT	250	-	-	100%
Androstenedione	10-20	30-45%	45-60%	10%
DHEA	5	80%	20%	-
DHEAS	Minima	>95%	<5%	-





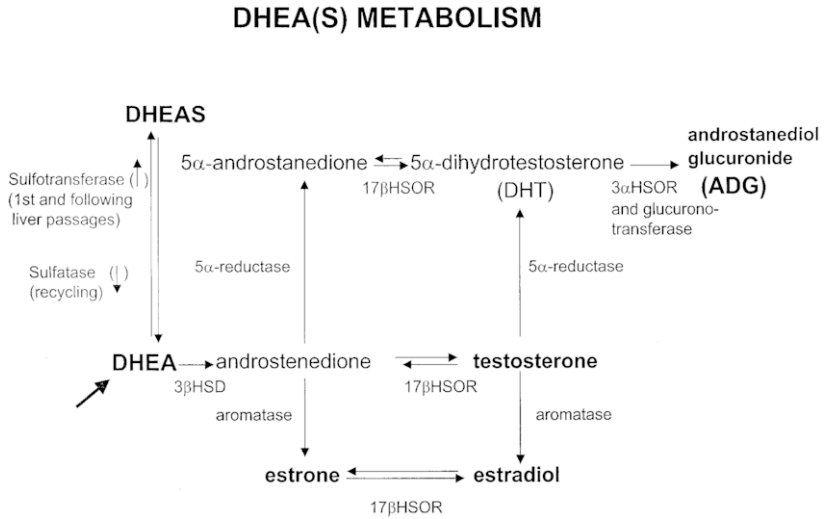
ANDROSTENEDIONE

	LIAISON ® Androstenedione	ACTIVE Androstenedione	DRG
Metodo	Competitive solid phase CLIA	competitivo RIA	competitivo ELISA
Sensibilità analitica:	0,17 ng/mL	0,05 ng/mL	0,04 ng/mL.
Precisione Intra-saggio	1-2,3%	7,5%	4.9-5.8%
Precisione Inter-saggio	2,4 – 9,7 %	11,3%	7.7-9.7%
Range di misura	0,24 e 10 ng/mL.	0,05 e circa 10,0 ng/mL.	0,04 e circa 10,0 ng/mL.
VR ng/mL.	*	M 0,62 – 3,12 F 0,24– 3,44 F menop 0,22 – 2,24	Maschi 0,3-2,4 Femmine 0,4-3,5
Cross-reazioni %	DHEA-S 15.000 ng/mL 0,002% Cortisolo 10.000 ng/mL 0,001% 17α-Idrossiprogesterone 10.000 ng/mL 0,006%	molto basse (Androsterone, 17-idrossiprogesterone, Cortisone, ecc.).	Androstenedione 100 DHEA 1,8 Testosterone 0,2 Estrone <0,1 Estradiolo <0,1 Progesterone <0,1 17OH Progesterone <0,1 5α-DHT <0,1 Cortisolo <0,01 DHEAs <0,01

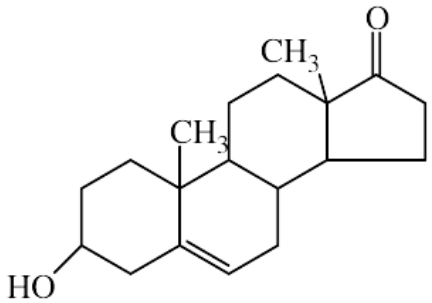
DEIDROEPIANDROSTERONE

⇒ Aumenti lievi o moderati sono spesso idiopatici

⇒ I metodi usati nei laboratori sono immunometrici diretti, che risentono delle interfenze



ORMONE	ATTIVITA' ANDROGENICA	ORIGINE Surrene	Ovaio	Periferia
Testosterone	100	5-25%	5-25%	50-70%
DHT	250	-	-	100%
Androstenedione	10-20	30-45%	45-60%	10%
DHEA	5	80%	20%	-
DHEAS	Minima	>95%	<5%	-



DEIDROEPIANDROSTERONE

Roche Diagnostics

Sostanza	Reattività crociata %	Concentraz. additiva µg/dL
Androstenedione	10.8	1000
DHEA	8.90	1000
Androsterone	2.10	2000
Testosterone	2.55	2000
Aldosterone	0.320	5000
Androsterone solfato	1.10	5000
DHEA-glucuronide	2.08	5000
Estradiolo	n. r.[d)]	5000
Estriolo	n. r.	5000
Estrone	0.740	5000
Estrone-3-solfato	0.500	5000
Progesterone	1.32	5000
5α-Diidrotestosterone	1.12	5000
19-Idrossiandrostendione	1.66	5000
Cortisolo	0.060	10000

Abbott

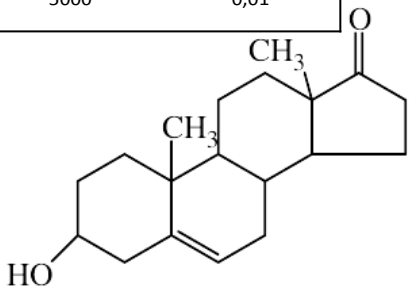
Sostanza	Concentrazione della sostanza a reattività crociata (µg/dl)	Reattività crociata (%)[a]
DHEA	4000	-0,002
Cortisolo	10000	0,000
Aldosterone	5000	-0,004
Estradiolo	5000	0,001
Testosterone	2000	0,000
5-diidrotestosterone	5000	-0,011
Androstenedione	1000	0,003
Androsterone	2000	-0,021
Andro-glucuronide	2000	-0,002
Estriolo	5000	0,008
Estrone	5000	0,001
19-idrossiandrostenedione	1000	0,025
Progesterone	5000	0,003
Androsterone solfato	5000	0,034
Estrone-3-solfato	5000	0,065
DHEA glucuronide	5000	0,006

SIEMENS

Composto	Quantità aggiunta (µg/dL)	% di reattività crociata
DHEA	4000	0,04
Aldosterone	5000	NRa
Androstenedione	1000	NR
Androsterone	2000	NR
Androsterone-glucuronide	5000	0,01
Cortisolo	10000	0,01
5-diidrotestosterone	5000	0,04
Estradiolo	5000	0,01
Estriolo	5000	0,01
Estrone	5000	0,01
Testosterone	2000	NR
19-idrossiandrostenedione	5000	0,04
Progesterone	5000	NR
B-estradiolo-3-solfato-17-glucuronide	5000	0,01

ANALYTICAL GOALS FOR INTERFERENCE

the maximum allowable systematic error produced by an interfering substance, should be: DHEAS 0,6%



Quando in una paziente con iperandrogenismo e sospetto di deficit late onset della 21 idrossilasi fareste il test con ACTH ?

A. Quando il valore basale fosse di 2 nmol/l

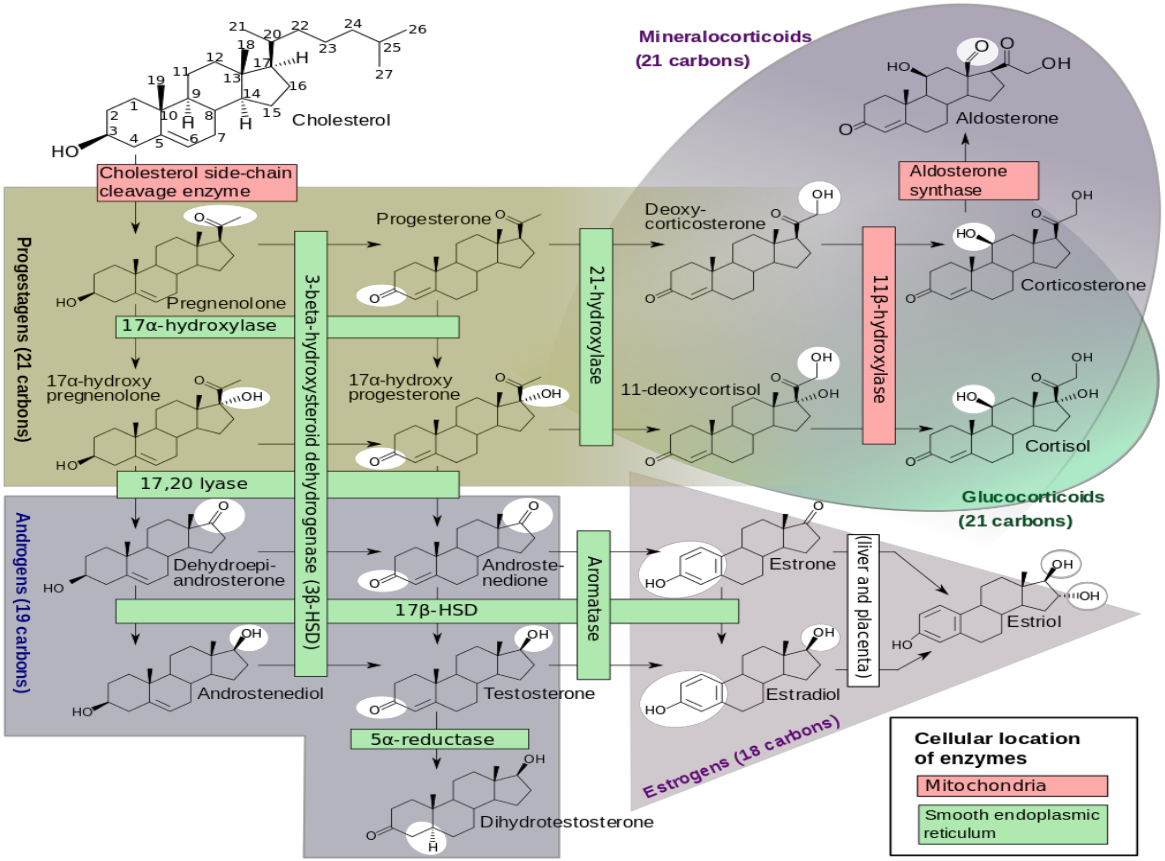
B. Quando valore basale fosse di 4 nmol/l

C. Quando valore basale fosse di 6 nmol/l

D. Quando il valore basale fosse di 20 nmol/l

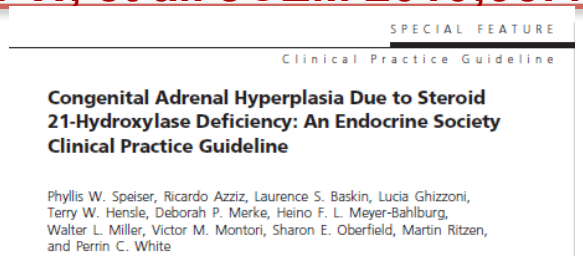
E. Se > 4 nmol/l MAI

17OHP and adrenal steroidogenesis



CAH ES guideline

Speiser PW, et al. JCEM 2010;95:4133-60



SPECIAL FEATURE

Clinical Practice Guideline

Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline

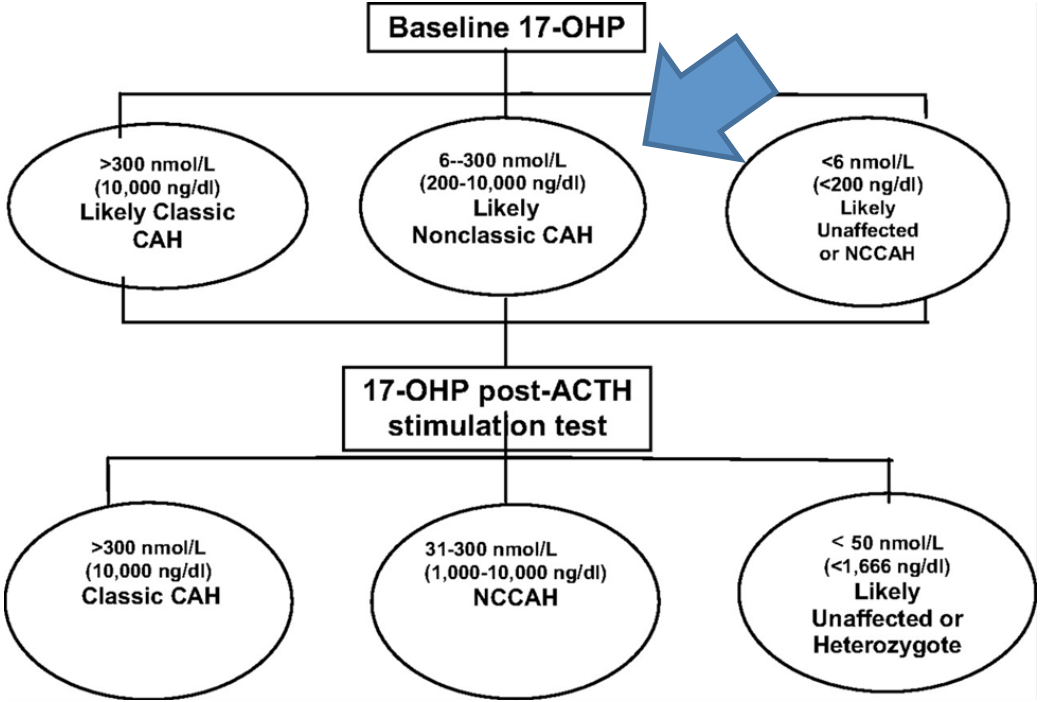
Phyllis W. Speiser, Ricardo Azziz, Laurence S. Baskin, Lucia Ghizzoni, Terry W. Hensle, Deborah P. Merke, Heino F. L. Meyer-Bahlburg, Walter L. Miller, Victor M. Montori, Sharon E. Oberfield, Martin Ritzen, and Perrin C. White

1.1 (Newborn screening). We recommend that screening for 21-hydroxylase deficiency … uses a two-tier protocol (initial immunoassay with further evaluation of positive test by liquid chromatography/tandem mass spectrometry)

3.1 (Diagnosis after infancy). We recommend obtaining an early morning basement serum 17-OHP in symptomatic individuals.

3.2. We recommend obtaining a complete adrenocortical profile after a cosyntropin stimulation test to make diagnosis in borderline cases

17-OHP screening



Hyperandrogenism/hirsutism

Martin KA, et al. JCEM 2018;103:1-25

CLINICAL PRACTICE GUIDELINE

Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society* Clinical Practice Guideline

Kathryn A. Martin,[1] R. Rox Anderson,[1] R. Jeffrey Chang,[2] David A. Ehrmann,[3] Rogerio A. Lobo,[4] M. Hassan Murad,[5] Michel M. Pugeat,[6] and Robert L. Rosenfield[3]

[1]Massachusetts General Hospital, Boston, Massachusetts 02114; [2]University of California, San Diego, La Jolla, California 92037; [3]University of Chicago, Chicago, Illinois 60637; [4]Columbia University, New York, New York 10032; [5]Mayo Clinic Evidence-Based Practice Center, Rochester, Minnesota 55905; and [6]Hospices Civils de Lyon, Bron, France F-69677

*Co-Sponsoring Associations: Androgen Excess and Polycystic Ovary Syndrome Society and European Society of Endocrinology.

1.2 We suggest screening hyperandogenemic women for NCCAH by measuring early morning 17-OHP levels in the follicular phase or on a random day….., even if TT or FT are normal

17-OHP assay methods

Manual (indirect, direct) Immunoassays

- Radio-immunoassay - RIA (since 1976)
- Enzyme-immunoassay- EIA (since 1978)

Automated, direct immunoassays

- Fluoro-immunoassay – FIA (since 1995)
- Chemiluminescence immunoassay - CLIA (since 2014)

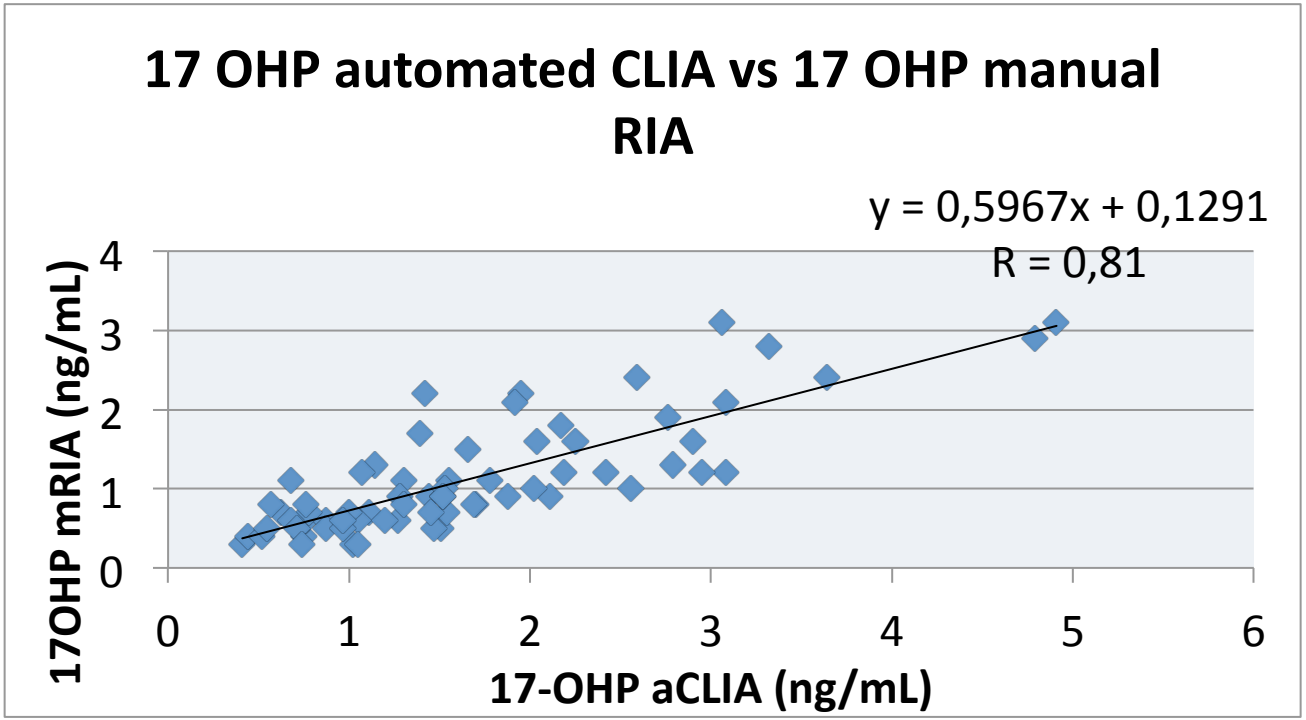
Liquid chromatography-tandem mass spectrometry (LC-MS) (since 2001)

17OHP immunoassays: quality specifications

Wudy SA, et al. The art of measuring steroids. J Steroid Biochem Mol Biol 2018;179:88-103

- Reference ranges adopted from the literature
- Measurement performed as single determination
- Adequate analytical sensitivity
- Poor analytical specificity
- Cross-reactivity
- Matrix effects

17OHP CLIA vs 17OHP RIA



Harmonization of serum/plasma 17 hydroxyprogesterone
Greaves RF, et al. CCLM 2018; eaop

- Disadvantages of immunoassays
  - Interference due to cross-reactivity, particurlarly 17α-hydroxypregnenolone sulfate (Wong, 1992)
  - No automation in RIA
- Advantages of immunoassays
  - Full automation in FIA and CLIA

Characteristics of commercially available automated IMAs

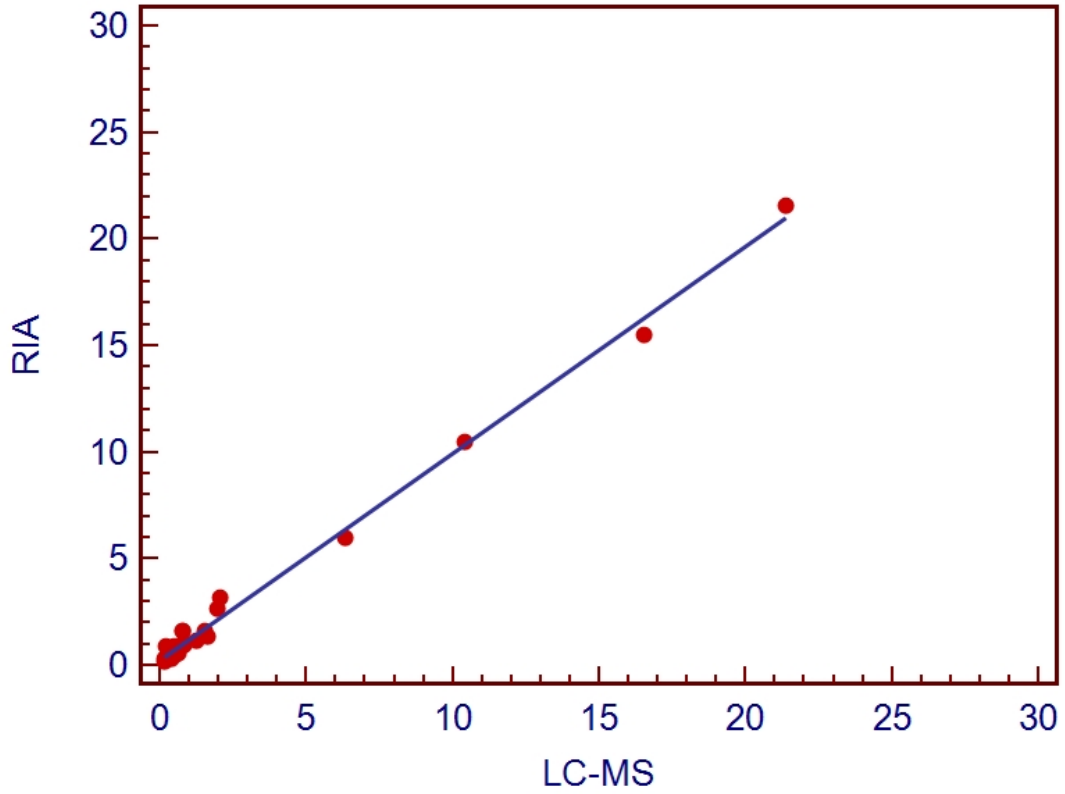
	SNIBE (Maglumi 2000 Plus)	IDS (IDS-iSYS)
Technology	CLIA	CLIA
Tracer	N-(aminobutil)-N-(ethyl)-isoluminol	Acridinium ester
Assay design	Competitive	Competitive
LoD*	0.1 ng/mL	0.15 ng/mL
LoQ*	Not declared	0.31 ng/mL
Measurement range	0.1 - 20 ng/mL	0.31 – 16 ng/mL
Calibration frequency	Every 4 weeks	Every 2 weeks
Time assay	25 minutes	40 minutes
Sample volume	40 µL	50 µL
Sample matrix	Serum	Serum, plasma (EDTA)

17OHP LC-MS/MS: characteristics

Wudy SA, et al. The art of measuring steroids. J Steroid Biochem Mol Biol 2018;179:88-103

- Simoultaneous measurement of several analytes (steroid profile)
- Effective and promising for second-tier testing, but also for first-tier testing
- High analytical specificity
- More expensive
- Time consuming
- High technical expertise

17-OHP: LC-MS/MS vs RIA



r: 0.997;
CL:
0.9934-0.99
87
P<0.0001

Take home messages

- For screening purposes of CAH, we recommend the use of two-tier protocol (initial immunoassay with further evaluation of positive test by LC-MS/MS) (Speiser, JCEM 2010)
- We recommend screening hyperandrogenic women for NCCAH by measuring early morning 17-OHP levels in the follicular phase or on a random day (Martin, JCEM 2018)
- A basal 17-OHP cut-off value of 2 ng/mL (6 nmol/L) is recommended for screening of NCCAH (Carmina, 2017)
- The ACTH test should be performed measuring 17-OHP 30 and/or 60 min after a 250 μg IV of cosyntropin (Carmina, 2017)
- Automated immunoassays are actually reliable (D'Aurizio, 2017).

LC-MS/MS is now performed in some specialty labs in current laboratory routine.