



SERVIZIO SANITARIO REGIONALE
EMILIA - ROMAGNA
Azienda Unità Sanitaria Locale di Bologna

Istituto delle Scienze Neurologiche
Istituto di Ricovero e Cura a Carattere Scientifico



Nuove applicazioni in spettrometria di massa

Laura Roli

Convegno Regionale SIBioC
Emilia Romagna 2019

**Presente e futuro
della medicina
di laboratorio**

Bologna
6 dicembre 2019 - 10:00 - 17:00

Oratorio di San Filippo Neri
via Manzoni 5



Caratteristiche delle tecniche di spettrometria di massa (MS)

sensibilità e specificità

intrinseche alla tecnologia:

- distinzione dei composti con similarità strutturale
- utilizzo di saliva e urina

possibilità di sviluppare pannelli multi-analita

su uno stesso campione:

- ampliamento del pannello delle molecole misurabili

precisione ed accuratezza

dovute all'impiego di standard interni marcati con isotopi stabili

flessibilità che consente l'analisi di molecole molto diverse tra loro sia per classe di appartenenza che per dimensioni

Limiti delle tecniche MS rispetto ai metodi immunochimici



Costo della tecnologia



Preparazione del campione



Competenza e formazione



Tempi di analisi

Round: IP425

LGC Standards Proficiency Testing

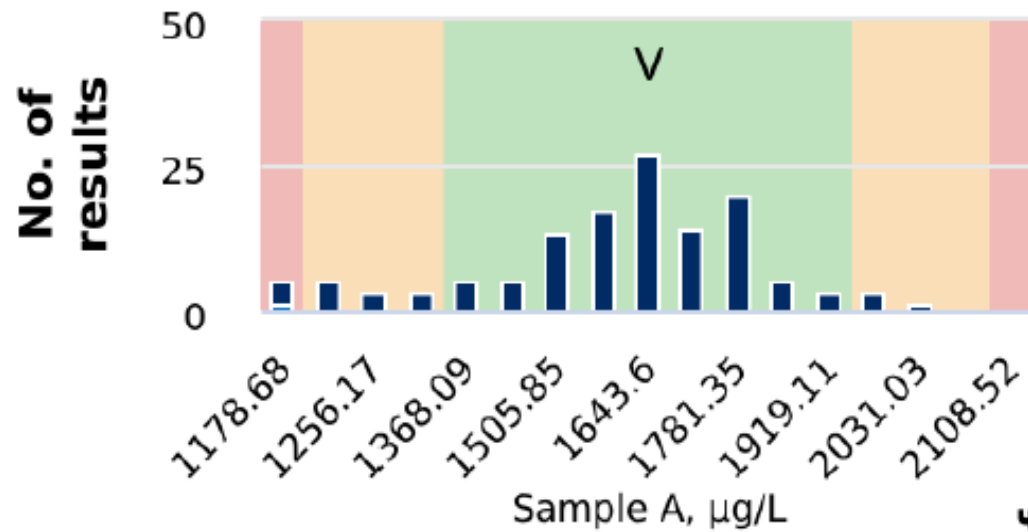
1 Chamberhall Business Park | Chamberhall Green | Bury | United Kingdom | BL9 0AP



Issue Number: 1

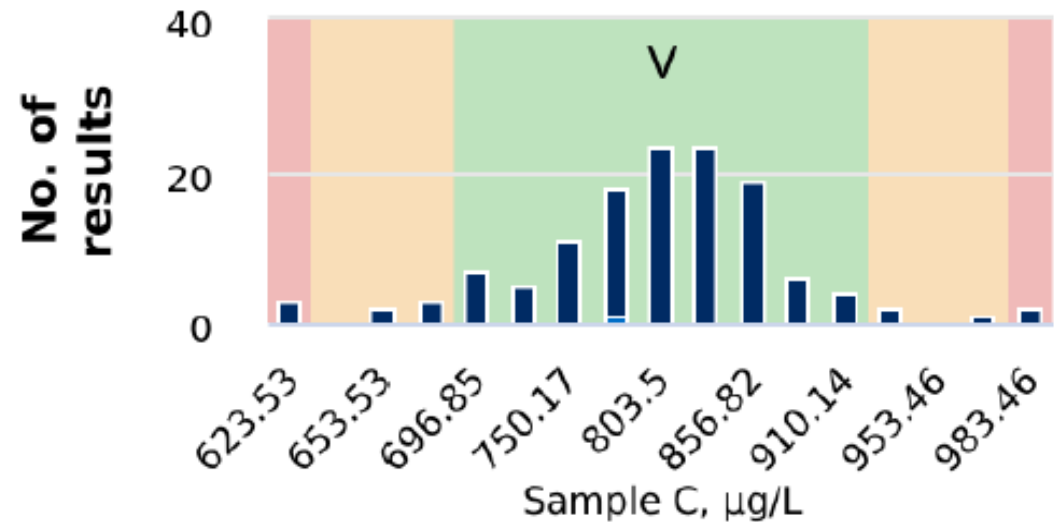
Issued: 10/09/2019

Sample	A	C
Satisfactory Range	1333.65 to 1953.55	683.52 to 923.47
Satisfactory z/z score	89.9%	89.9%
Questionable z/z score	9.2%	6.2%
Unsatisfactory z/z score	0.8%	3.9%



Sample A:
Ciclosporin spike value 1500.08 µg/L

Sample C:
Ciclosporin spike value 699.77 µg/L



Results for major method groups nmol/L (ng/mL)

Sample	Method	n	Method Mean	SD	CV
553	(all methods)	661	49.3 (19.7)	6.3 (2.5)	12.9
553	Abbott Alinity i	10	45.8 (18.3)	1.8 (0.7)	4.0
553	Abbott Architect - New (5P02)	48	45.7 (18.3)	2.2 (0.9)	4.9
553	Beckman Access2 Total 25OHD	3	46.9 (18.8)	4.5 (1.8)	9.5
553	Beckman Unicel Dxi Total 25OHD	25	47.9 (19.2)	8.5 (3.4)	17.8
553	DiaSorin Liaison Total	183	48.9 (19.6)	4.4 (1.8)	9.0
553	HPLC	12	50.2 (20.1)	18.5 (7.4)	36.9
553	IDS-iSYS	27	54.2 (21.7)	5.1 (2.1)	9.5
553	IDS-iSYS New	18	56.4 (22.6)	4.6 (1.8)	8.1
553	LC-MS-MS	141	48.9 (19.6)	6.5 (2.6)	13.3
553	Roche Total 25OHD	58	55.0 (22.0)	8.9 (3.6)	16.2
553	Roche Vitamin D Total II	56	45.9 (18.4)	3.5 (1.4)	7.6
553	Siemens ADVIA Centaur	35	51.3 (20.5)	6.5 (2.6)	12.6
553	Target Value	50.2	nmol/L		
553	Target Value Uncertainty	0.6	nmol/L		

Target values for 25-OHD2 and 25-OHD3 provided by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA using their isotope dilution LC-MS/MS Reference Measurement Procedure.

Data elaborazione: 01/03/2017

Analita	Unità	Campione	HPLC-SM	Consenso	CVt%	Differenza
Cortisolo (*)	µg/L	IM71a	34	35,6	13%	+5%
		IM71c	227	225	10%	-1%
Progesterone (*)	µg/L	IM71a	0,584	0,68	27%	+16%
		IM71c	16,6	19,1	15%	+15%
Testosterone (***)	µg/L	IM71a	0,85	0,75	22%	-12%
		IM71c	2,1	1,93	11%	-8%
Aldosterone (***)	ng/L	IH71a	66	64	26%	-3%
		IH71b	322	291	19%	-10%
delta-4-Androstenedione (***)	µg/L	IH71a	0,43	0,61	52%	+42%
		IH71b	2,4	3,2	24%	+33%
17alfa-OH-progesterone (***)	µg/L	IH71a	0,76	0,85	32%	+12%
		IH71b	7,8	5,6	30%	-28%
25(OH) vitamina D (***)	µg/L	IV71a	16,2	16	30%	-1%
		IV71b	106	71,5	17%	-33%
25(OH) vitamina D (**)	µg/L	IV71a	14,8	16	30%	+8%
		IV71b	103	71,5	17%	-31%

Valori ottenuti con la tecnica cromatografia liquida/spettrometria di massa (HPLC-SM) per gli ormoni steroidei nei campioni IM71a/c e IH71a/b e per la 25(OH) vitamina D nel campione IV71a/b: nella tabella seguente si riportano i valori di HPLC-SM confrontati con le medie di consenso. Sono riportate anche le variabilità totali (CVt%) osservate nei campioni di controllo che rappresentano un indice dell'accordo tra metodi. Si deve tenere presente che quando le differenze tra metodi sono elevate i risultati di HPLC-SM devono essere confrontati con le medie dei singoli metodi.



Review

Systematic review of serum steroid reference intervals developed using mass spectrometry

Nevada Tavita ^a, Ronda F. Greaves ^{a, b}  

After accounting for duplicates, a total of 60 manuscripts were identified through the search strategy. Following critical [evaluation](#), a total of 16 studies were selected. Of the 16 studies, 12 reported reference intervals for [testosterone](#), 11 for [17 hydroxy-progesterone](#), nine for [androstenedione](#), six for [cortisol](#), three for [progesterone](#), two for [dihydrotestosterone](#) and only one for [aldosterone](#) and [dehydroepiandrosterone sulphate](#). No studies established MS-based reference intervals for [oestradiol](#).

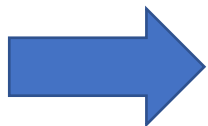
Review

Systematic review of serum steroid reference intervals developed using mass spectrometry


Nevada Tavita ^a, Ronda F. Greaves ^{a, b}  

Highlights

- A systematic review of the English language literature looked for mass spectrometry based serum steroid reference intervals.
- Sixteen peer reviewed publications have developed reference intervals in healthy cohorts.
- Published reference intervals for 17 serum/plasma steroids were identified.
- Reporting units, age stratification and reference intervals are inconsistent between publications for individual steroids.
- The task of applying universally applicable reference intervals is challenging.



The steroid response to human chorionic gonadotropin (hCG) stimulation in men with Klinefelter syndrome does not change using immunoassay or mass spectrometry

L. Roli¹ · D. Santi^{2,3}  · S. Belli² · S. Tagliavini¹ · S. Cavalieri⁴ · M. C. De Santis¹ · E. Baraldi¹ · F. Fanelli⁵ · M. Mezzullo⁵ · A. R. Granata³ · U. Pagotto⁵ · R. Pasquali⁵ · V. Rochira^{2,3} · C. Carani² · M. Simoni^{2,3,6} · T. Trenti¹

Results 13 KS patients (36 ± 9 years) not receiving *T* replacement therapy and 14 controls (32 ± 8 years) were enrolled. *T*, progesterone, cortisol, 17-hydroxy-progesterone (17OHP) and androstenedione, were significantly higher using IAs than LC-MS/MS. IAs and LC-MS/MS showed direct correlation for all five steroids, although the constant overestimation detected by IAs. Either methodology found the same 17OHP and *T* increasing profile after hCG stimulation, with equal areas under the curves (AUCs).

MS QUANDO?

«L'AUMENTO DELLA SENSIBILITÀ E LA RIDUZIONE DELLA VARIABILITÀ ANALITICA RAPPRESENTANO VALORI ESSENZIALI»



**1° esempio:
ipotiroidismo
subclinico**

A pilot study: Subclinical hypothyroidism and free thyroid hormone measurement by immunoassay and mass spectrometry

Verena Gounden ^a, Jacqueline Jonklaas ^b, Steven J. Soldin ^{a, b}  

Results

Sixty five percent ($n = 26$) of patients ($n = 40$) had (LC-MS/MS) FT4 or FT3 or both FT4 and FT3 values below mass spectrometry reference limits.

Conclusions

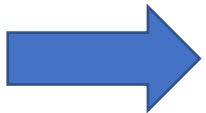
Our findings indicate that the direct analogue immunoassay method for FT4 measurement results in a significant proportion of patients being misclassified as having subclinical hypothyroidism.

A pilot study: Subclinical hypothyroidism and free thyroid hormone measurement by immunoassay and mass spectrometry

Verena Gounden ^a, Jacqueline Jonklaas ^b, Steven J. Soldin ^{a, b}  

Highlights

- MS analysis of outpatient samples with elevated TSH and normal immunoassay FT4 values was performed.
- This study revealed that 65% of these patients had low FT4 values by LC-MS/MS measurement.
- MS analysis of free thyroid hormones is recommended where subclinical hypothyroidism is suspected.
- Consider MS analysis of FT4 & FT3 when immunoassay results are discrepant to TSH/clinical findings.



MS QUANDO?

«L'AUMENTO DELLA SENSIBILITÀ E LA RIDUZIONE DELLA VARIABILITÀ ANALITICA RAPPRESENTANO VALORI ESSENZIALI»



**2° esempio:
deficit di
testosterone**



Original Research

Endocrine

Testosterone Deficiency: A Review and Comparison of Current Guidelines

Jethro C.C. Kwong BMSc¹, Yonah Krakowsky MD², Ethan Grober MD²  

Results: Guidelines from the Canadian Medical Association Journal, American Urological Association, European Association of Urology, Endocrine Society, International Society for Sexual Medicine, and British Society for Sexual Medicine were included for review. Recommendations were generally consistent across guidelines. Key differences include the biochemical cutoff for low T, and recommendations for patients with low to normal T, prostate cancer, or cardiovascular disease. We highlight several case scenarios in which management differs depending on the guideline adopted.

Table 1. Current guidelines for the diagnosis and management of TD

	Topic	ISSM (2015)	CMAJ (2015)	BSSM (2017)	AUA (2018)	ES (2018)	EAU (2018)	
Evaluation	Definition of TD	Signs and symptoms of TD with low TT						
	Biochemical cutoff for low TT	<231 ng/dL (<8 nmol/L); 2 measurements	No specific cutoff; 1 measurement	<231 ng/dL (<8 nmol/L); 2 measurements	<300 ng/dL (<10.4 nmol/L); 2 measurements	<264 ng/dL (<9.2 nmol/L); 2 measurements	<231 ng/dL (<8 nmol/L); 2 measurements	
Management	Principles of management	Symptomatic improvement with minimal side effects. Shared decision making in setting of comorbidities. Include lifestyle modifications and optimize comorbidities. Consider contraindications to TTh. Shared decision making in setting of comorbidities. Consider contraindications to TTh.						
	Target T range for TTh	Mid-normal	404–505 ng/dL (14–17.5 nmol/L)	404–505 ng/dL (14–17.5 nmol/L)	450–600 ng/dL (15.6–20.8 nmol/L)	350–600 ng/dL (14.1–24.5 nmol/L)	Mid-normal	

AUA = American Urological Association; BSSM = British Society for Sexual Medicine; CMAJ = Canadian Medical Association Journal; CVD = cardiovascular disease; DRE = digital rectal exam; EAU, European Association of Urology; ES = Endocrine Society; FSH = follicle-stimulating hormone; FT = free testosterone; GHG = gonadotropin-releasing hormone; IPSS = International Prostate Symptom Score; ISSM = International Society for Sexual Medicine; MI = myocardial infarction; NYHA = New York Heart Association; LH = luteinizing hormone; LUTS = lower urinary tract symptoms; OSA = obstructive sleep apnea; PCa = prostate cancer; PRL = prolactin; PSA = prostate-specific antigen; SHBG = sex hormone-binding globulin; TD = testosterone deficiency; TT = total testosterone; TTh = testosterone therapy.

	Topic
Evaluation	Biochemical cutoff for low TT
Management	Target T range for TTh

All guidelines agree that liquid chromatography/mass spectrometry (LCMS) is the gold standard for TT measurement but also recognize that this might not be feasible at every institution. The CMAJ, BSSM, ES, and EAU recommend the use of validated immunoassays if LCMS is not available. The ES specifies the use of a harmonized reference range for immunoassays certified by the Centers for Disease Control and Prevention Hormone Standardization Program for Testosterone. In contrast, the EAU recommends using laboratory-specific reference ranges, whereas the AUA and BSSM advise using the absolute TT value.

MS QUANDO?

«L'AUMENTO DELLA SENSIBILITÀ E LA RIDUZIONE DELLA VARIABILITÀ ANALITICA RAPPRESENTANO VALORI ESSENZIALI»



**3° esempio:
PCOS**

Implications of Androgen Assay Accuracy in the Phenotyping of Women With Polycystic Ovary Syndrome FREE

Flavia Tosi, Tom Fiers, Jean-Marc Kaufman, Marlene Dall'Alda, Rossella Moretta, Vito Angelo Giagulli, Enzo Bonora, Paolo Moghetti ✉

The Journal of Clinical Endocrinology & Metabolism, Volume 101, Issue 2, 1 February 2016, Pages 610–618, <https://doi.org/10.1210/jc.2015-2807>

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ENDOCRINOLOGY
& METABOLISM

Results:

By using gold standard assays, TT was high in 36.3% of women, whereas A only marginally contributed to identifying hyperandrogenemic patients. However, gold standard FT measurement was elevated in 70.6% of the PCOS patients, identifying them as hyperandrogenemic. Routine TT and A assays, and the derived calculated FT, were strikingly inaccurate, with substantial overestimation. These assays erroneously classified 60 patients (29.4%), 32 as false hyperandrogenemic, and 28 as false normoandrogenemic, with incorrect assignment of many patients to the clinical phenotypes of PCOS and inappropriate estimation of their metabolic risk. In particular, women misclassified as normoandrogenic had a more severe metabolic profile than true normoandrogenic subjects.

Applicazioni MS per la medicina di laboratorio

Endocrinologia	Steroidi, loro precursori e metaboliti (Protocollo per l'iperplasia surrenale congenita (17-OHP, Cortisolo, Androstenedione))
	Amine biogene e loro metaboliti
	<i>Ormoni tiroidei liberi, Angiotensina II, hGH e IGF1 e binding proteins</i>
Monitoraggio terapeutico dei farmaci	Immunosoppressori (Ciclosporina, Sirolimus, Everolimus, Tacrolimus)
	Antiepilettici (Acido Valproico, Fenobarbital, Topiramato, Vigabatrim, Levetiracetam)
	Antitumorali (Vincristina, Ciclofosfamide, Metotrexate, ...)
	antiretrovirali
	anticoagulanti

Applicazioni MS per la medicina di laboratorio

Altre applicazioni	Aminoacidi ed acidi organici, acidi grassi
	Acidi biliari, e loro coniugati
	Emoglobina, Emoglobina Glicata e varianti dell'Emoglobina
	Vitamine (Gruppo B, D)
	Droghe d'abuso
	Biomarcatori di abuso alcolico (EtG, EtS, CDT)

Microbiologia diagnostica	identificazione di batteri con pochi tratti fenotipici caratteristici, funghi
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Verso il futuro



La spettrometria di massa in genomica

L'analisi dei geni a livello degli oligonucleotidi per la caratterizzazione di singoli polimorfismi del nucleotide e ripetizioni tandem brevi

L'analisi a livello delle proteine ha come target la funzione dei geni o “genomica funzionale”

Caratterizzazione delle proteine

- *Interazioni tra proteine*
- *Espressione di proteine*
- *Modificazioni delle proteine*

La MS nella Identificazione di biomarcatori proteici di malattia

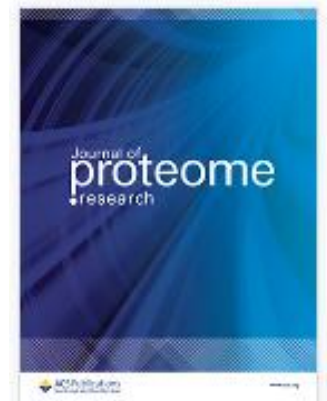
- ❑ **Cancro alla prostata** (Fujita K. 2018; Wu D. 2017; Tanase CP. 2017; Flores-Morales A. 2017; Flatley B. 2014)
- ❑ **Tumore ovarico** (Swiatly A. 2018; Sadłeckki P. 2017; Acland M. 2018; Banach. P 2017)
- ❑ **Tumore mammella** (Nagini S. 2017; Mueller C. 2018; Banach P. 2017, Zhang B. 2019)
- ❑ **Mieloma** (Thoren KL. 2018)
- ❑ **Tumore pancreatico** (Kim H. 2017; Pan S. 2019)

Reproducibility of Mass Spectrometry Based Protein Profiles for Diagnosis of Breast Cancer across Clinical Studies: A Systematic Review

Anne K. Callesen^{*†‡}, Werner Vach[§], Per E. Jørgensen[‡], Søren Cold^{||}, Ole Mogensen⁺, Torben A. Kruse[‡], Ole N. Jensen^{*†} and Jonna S. Madsen[‡]

[...] despite known problems related to reproducibility, we were able to demonstrate overlap in peaks between clinical studies indicating some convergence toward a set of common discriminating, reproducible peaks for breast cancer. These peaks should be further characterized for identification of the protein identity and validated as biomarkers for breast cancer

J Proteome Res. 2008, 7, 4, 1395-1402



Journal of Proteome
Research

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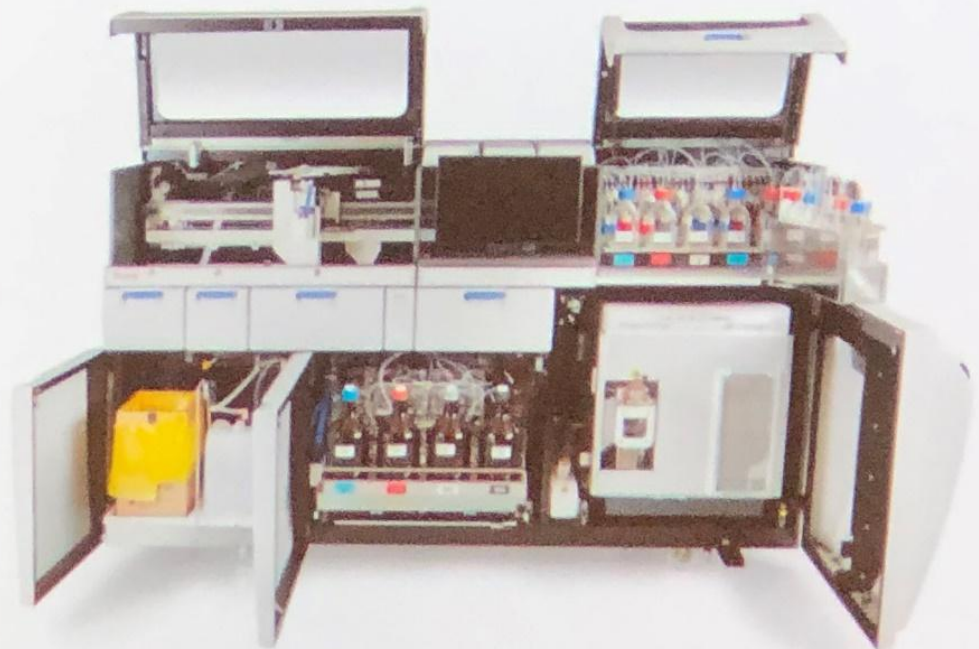
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