Benefits of LC-MS/MS

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Lunch and Learn, Roche,2025 June 10th

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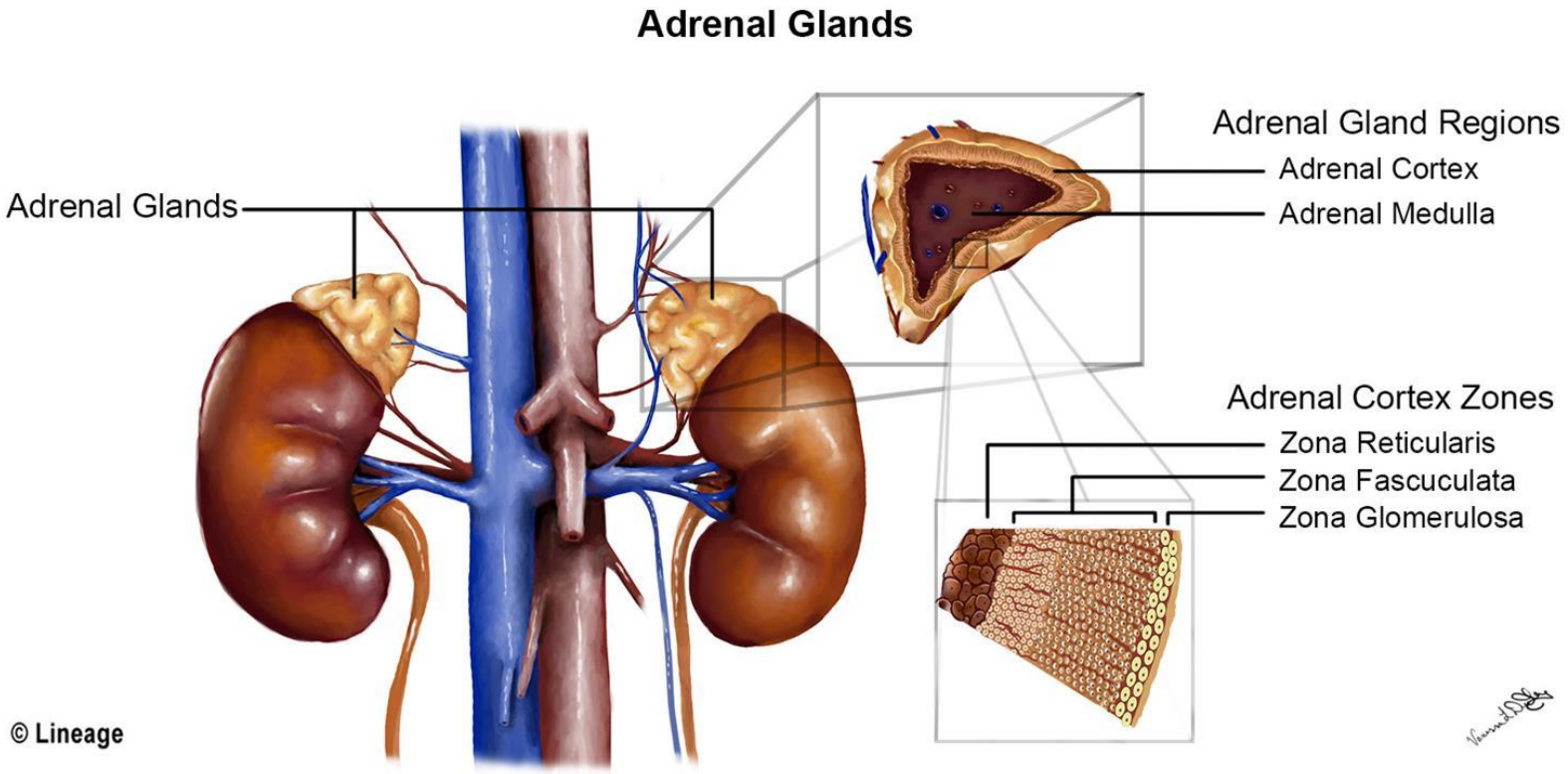
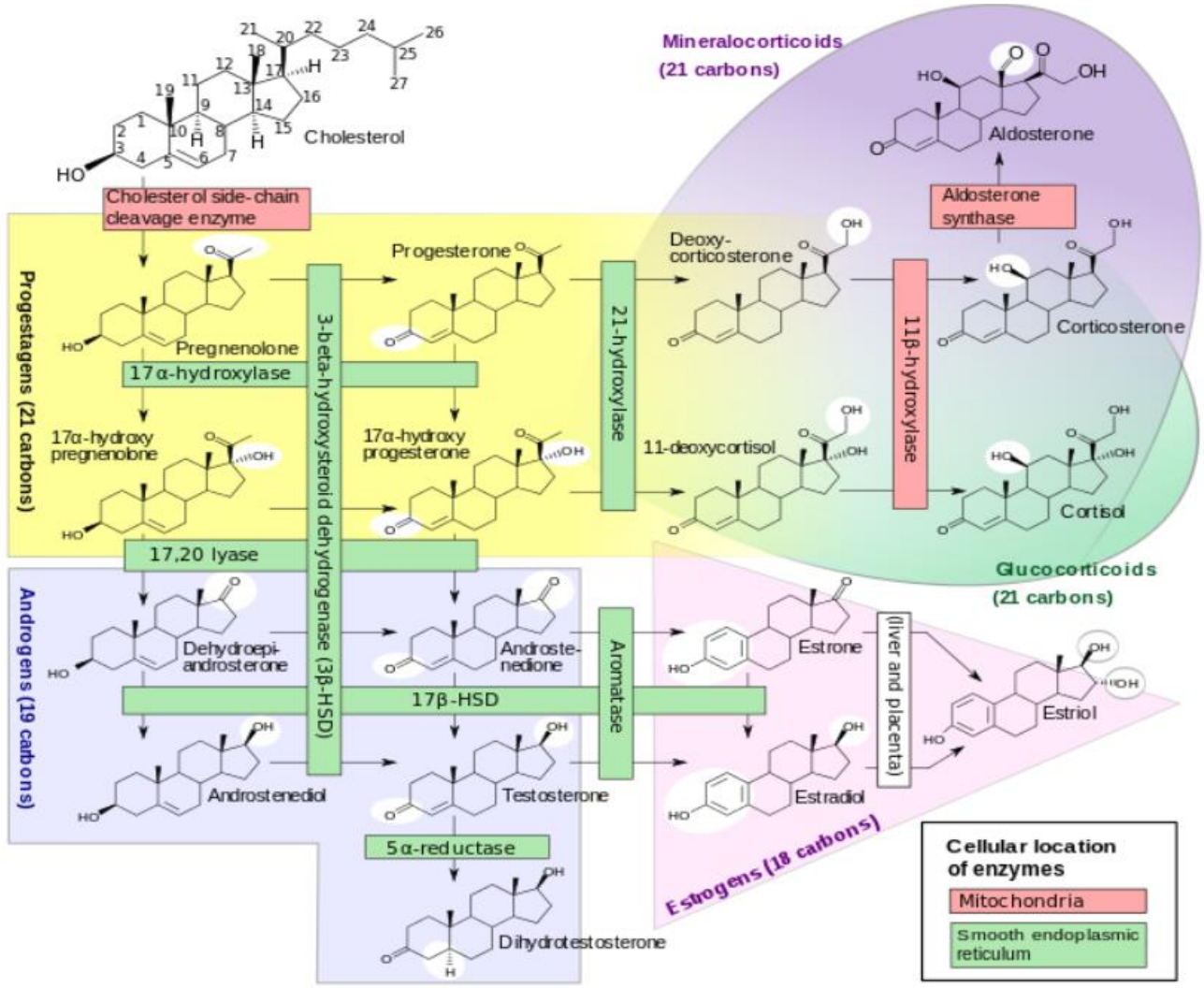
Steroid hormones



Source: https://step1.medbullets.com/endocrine/109001/adrenal-cortex-and-medulla

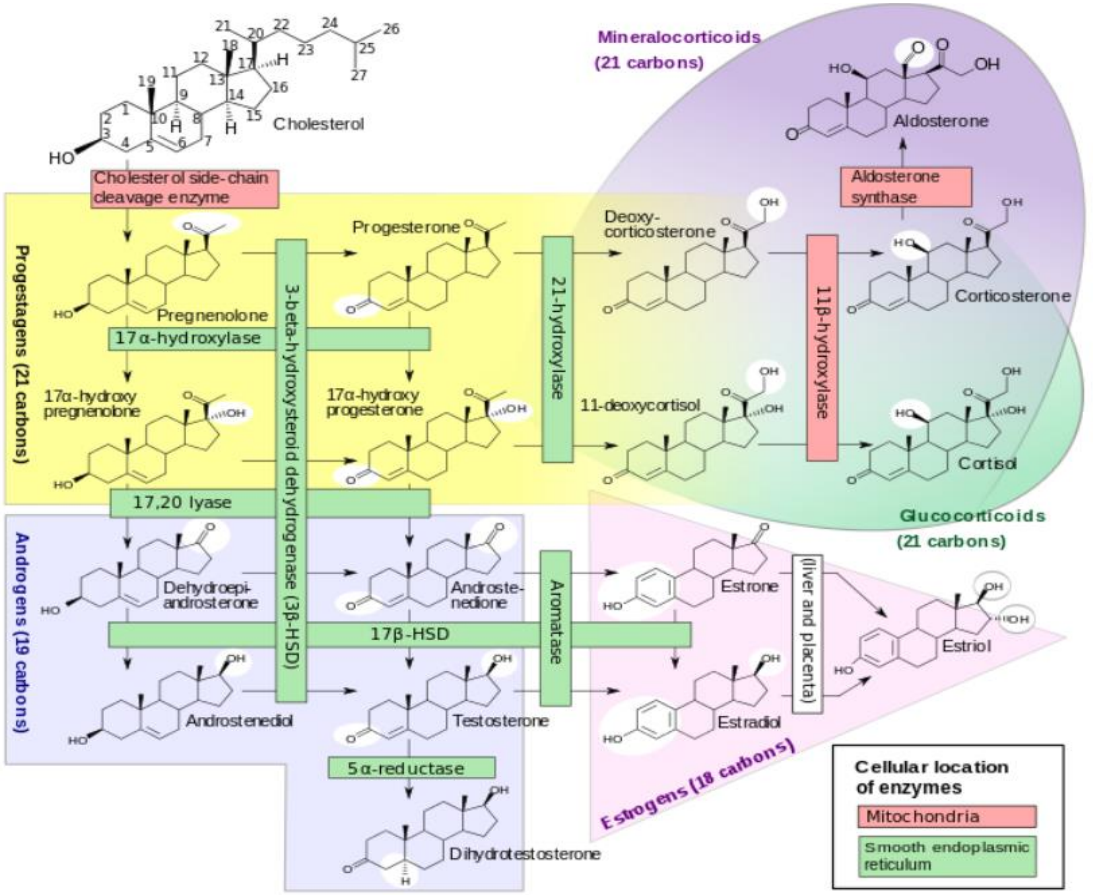
Steroid hormones



Steroid hormones

Crucial role : control of

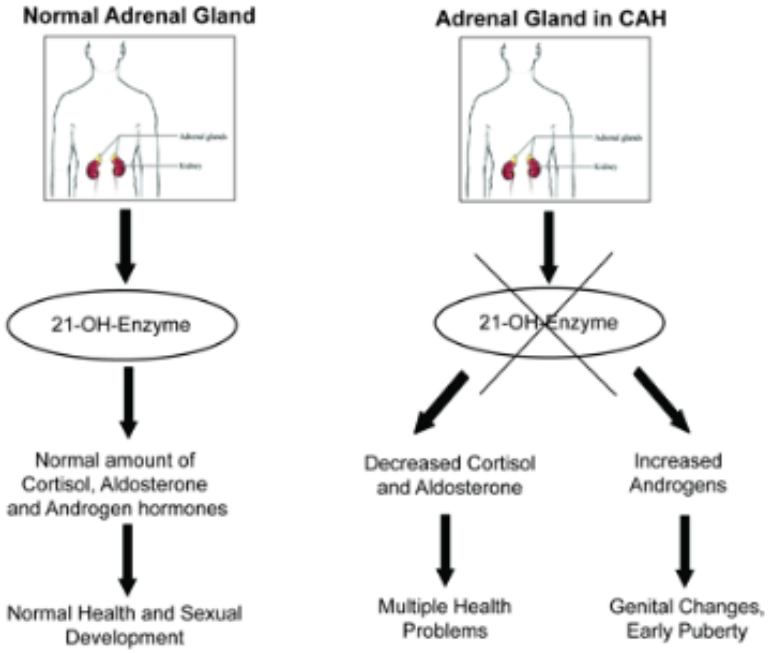
- metabolism
- immune functions
- inflammation



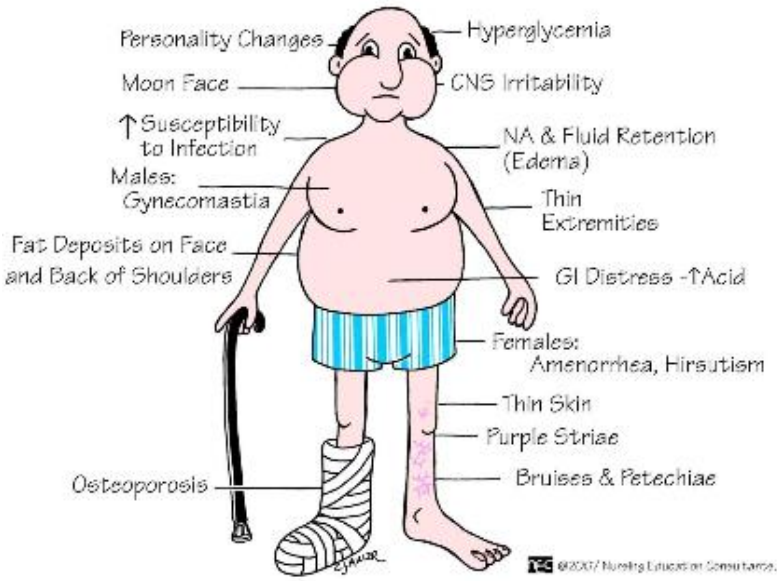
Steroid hormones

Modifications in steroid profiling reflect disease status and help research into a wide number of health disorders.

Congenital Adrenal Hyperplasia (CAH)



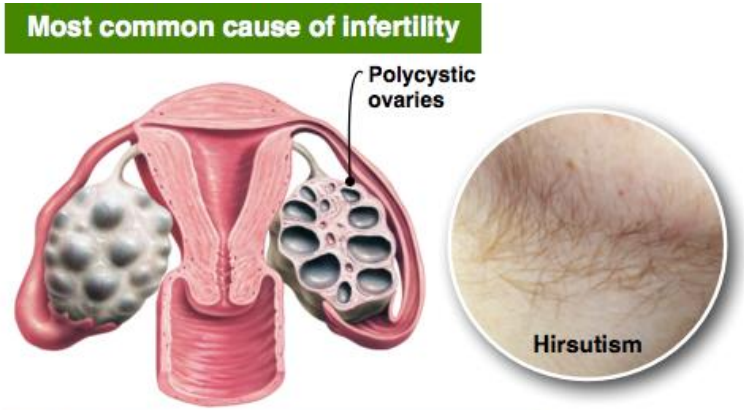
Cushing's syndrome



Steroid hormones

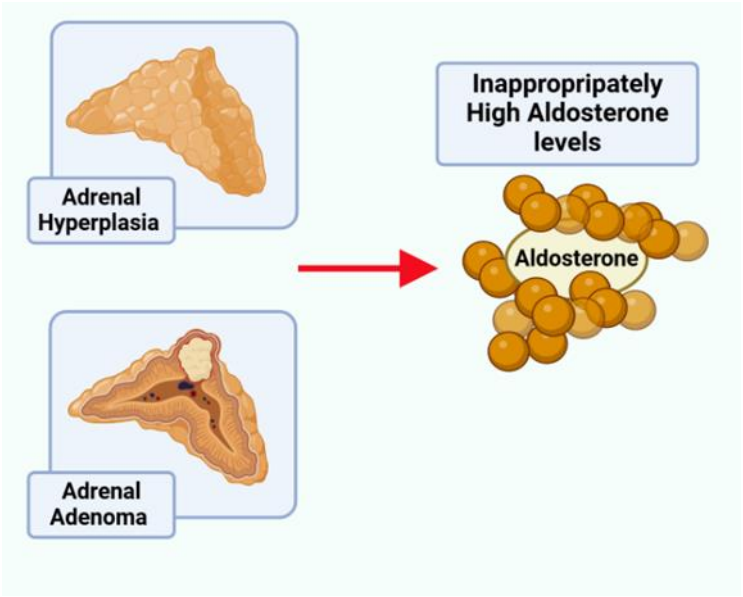
Modifications in steroid profiling reflect disease status and help research into a wide number of health disorders.

Polycystic Ovarian Syndrome



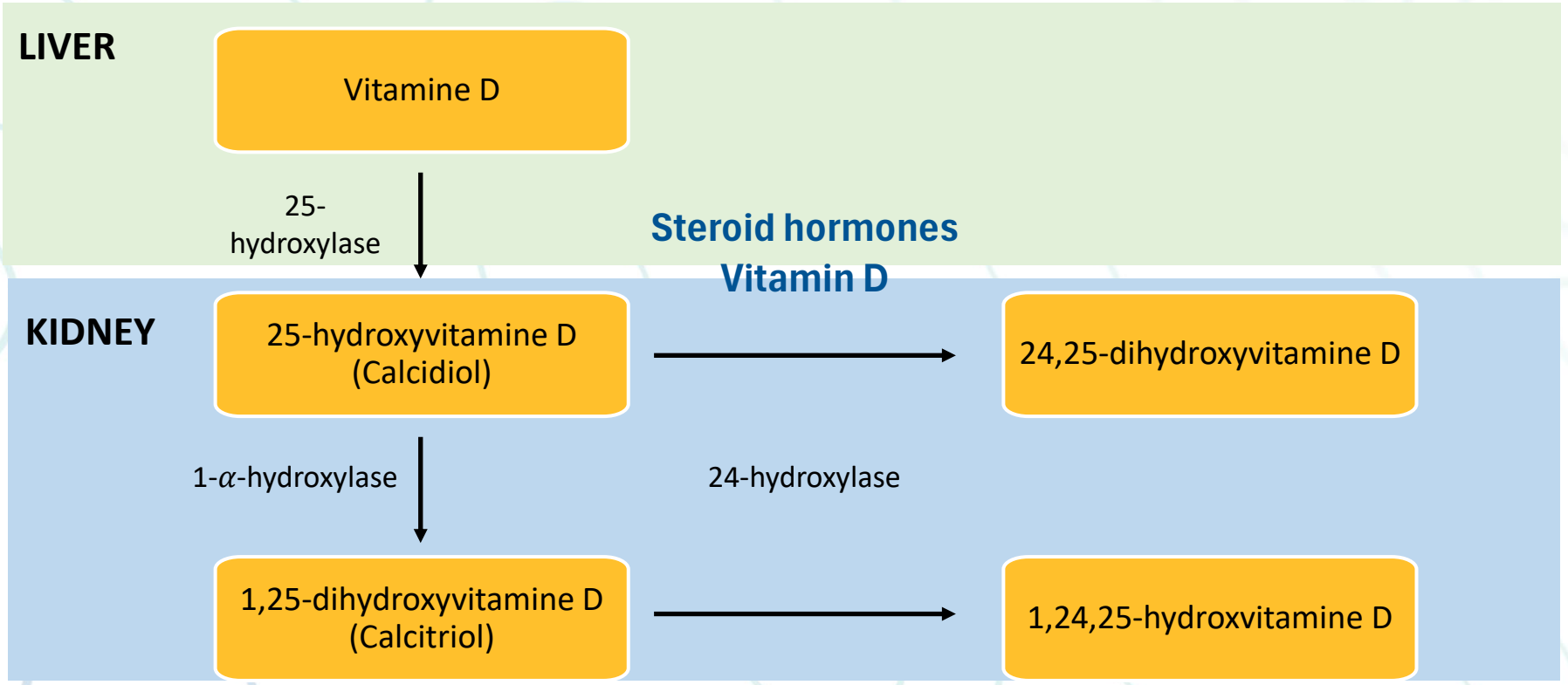
Clinical
- Hirsutism
- Menstrual irregularities
- Acanthosis nigricans
- Obesity
- Acne
- Insulin resistance and metabolic syndrome
- Sleep-disordered breathing
- Fatty liver disease

Hyperaldosteronism



Steroid hormones
Vitamin D

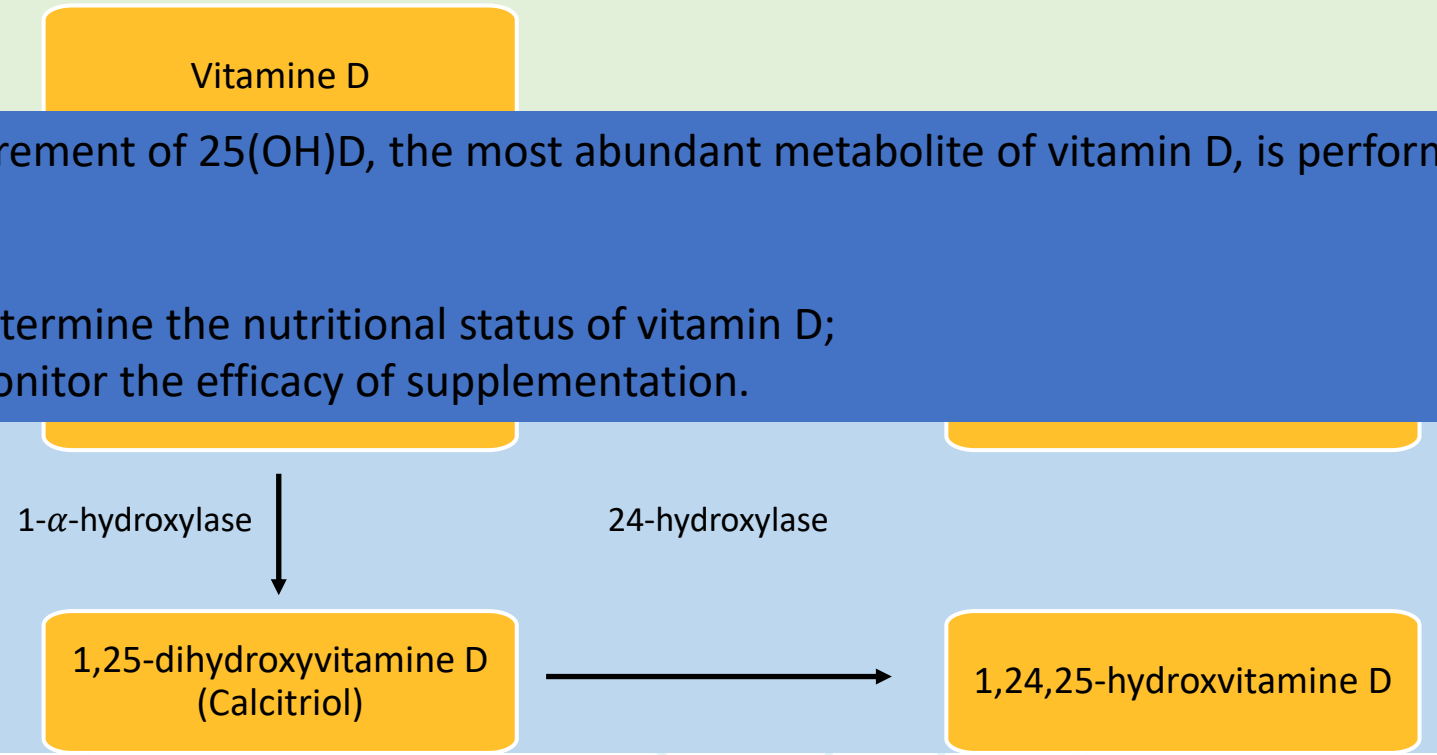
Metabolisation



Steroid hormones
Vitamin D

Metabolisation

LIVER



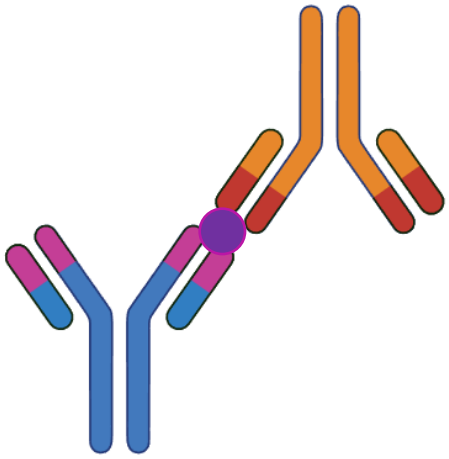
The measurement of 25(OH)D, the most abundant metabolite of vitamin D, is performed mainly:

1. to determine the nutritional status of vitamin D;
2. to monitor the efficacy of supplementation.

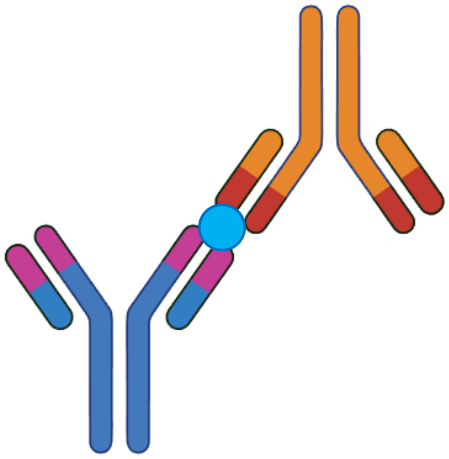
Steroid hormones

Quantification of steroids by immunoassays

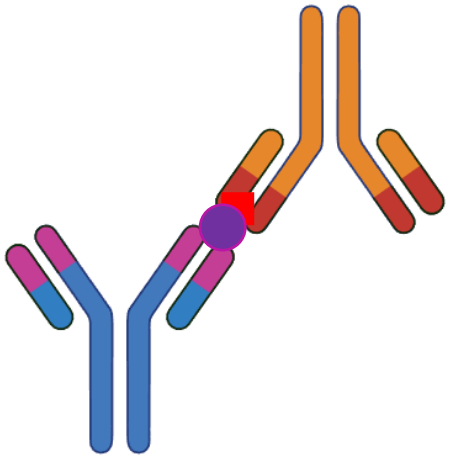
Specific interaction



Cross-reactivity



Interference



Steroid hormones

Interference Type	Mechanism	Impact	Detection/Prevention Strategies
Heterophile Antibodies	Endogenous antibodies that cross-link assay antibodies, leading to non-specific binding.	Falsely elevated or decreased hormone levels.	Use of heterophile-blocking reagents; alternative assay platforms; sample dilution tests.
Human Anti-Animal Antibodies (HAAA)	Develop in patients exposed to animal-derived products; interfere with assay antibodies.	Erroneous hormone measurements.	Pre-treatment with blocking agents; employing assays less susceptible to HAAA interference.
Anti-Analyte Antibodies	Patient-generated antibodies against specific hormones, altering assay binding.	Inaccurate hormone quantification.	Alternative measurement methods; immunoglobulin precipitation techniques.
Biotin Interference	High levels of biotin from supplements disrupt biotin-streptavidin assay systems.	Falsely high or low results, depending on assay design.	Patient history assessment; using biotin-free assays; delaying sample collection post-biotin ingestion.
Cross-Reactivity	Assay antibodies bind to structurally similar non-target molecules.	Overestimation of hormone levels.	Utilization of highly specific antibodies; confirmatory testing with alternative methods.

Adapted from https://doi.org/10.3343/alm.2022.42.1.3

Steroid hormones

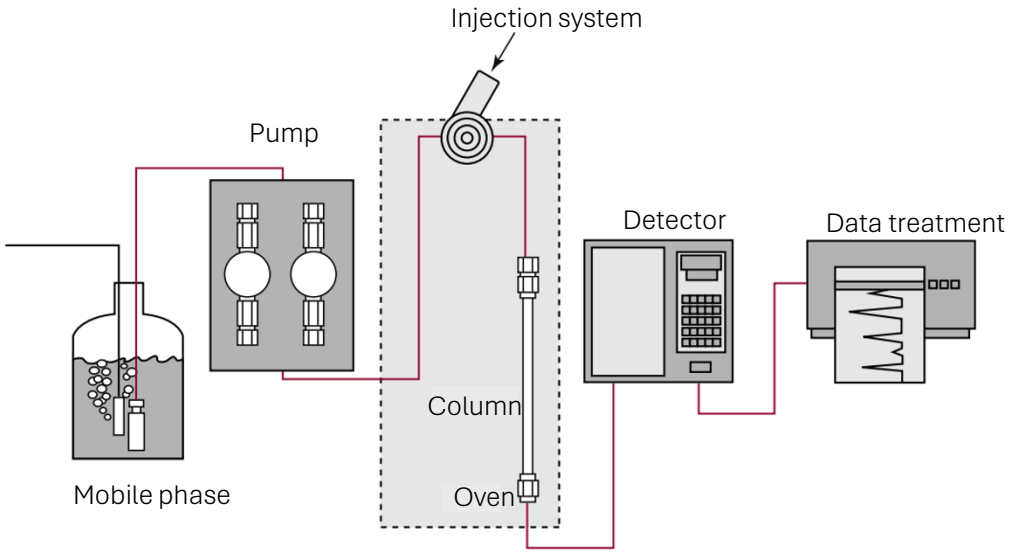
Interference Type	Mechanism	Impact	Detection/Prevention Strategies
Hook Effect	Extremely high antigen levels saturate antibodies, preventing proper sandwich formation.	Falsely low hormone readings.	Serial sample dilutions; awareness of clinical context suggesting high hormone levels.
Endogenous Antibodies	Autoantibodies or paraproteins interfere with assay components.	Variable effects on assay accuracy.	Immunoglobulin removal techniques; alternative assay methodologies.
Preanalytical Factors	Sample handling issues like hemolysis, lipemia, or improper storage.	Erroneous hormone measurements.	Strict adherence to sample collection and handling protocols; visual inspection of samples.
Matrix Effects	Variations in sample composition affect assay performance.	Inconsistent or inaccurate results.	Matrix-matched calibrators; validation of assay performance across different sample types.
Exogenous Substances	Medications or supplements that interfere with assay reactions.	Altered hormone readings.	Comprehensive patient medication history; selecting assays with minimal known interferences.

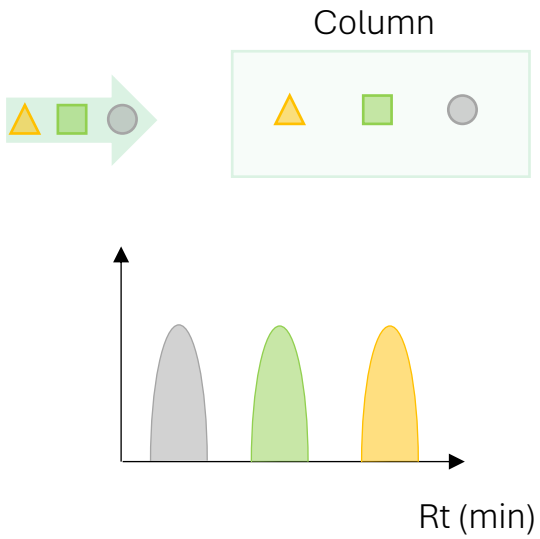
Adapted from https://doi.org/10.3343/alm.2022.42.1.3

LC-MS/MS

1. Liquid chromatography

Separation technique of analyte based on the affinity for a stationary phase and a mobile phase.





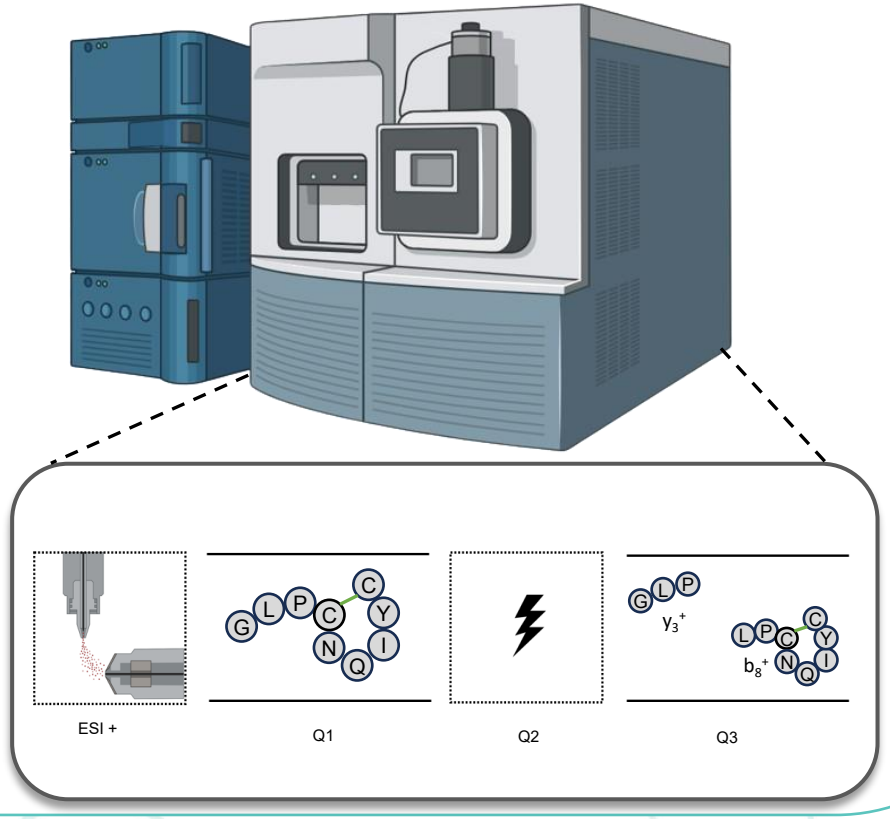
LC-MS/MS

2. Mass spectrometry

Analytical technique that ionizes chemical species contained in a sample and analyzes the produced ions in the gas phase.

Mass spectrometer

- Ion source (ionization of sample)
- Analyzer (*m/z* separation in time or in space)
- Detector (ion detection and generation of the mass spectrum)



Why LC-MS/MS?

Table 1. Common problems of cross-reaction and specificity in current immunoassays

Analyte	Cross-reacting compound	Context	Reference
Competitive immunoassays			
17OH progesterone	17OH pregnenolone sulfate	Neonates (especially preterm)	Wong, *et al.* 1992 [182] Makela and Ellis, 1988 [183]
Cortisol	11 desoxy cortisol	11 Hydroxylase defect Metyrapone test	Ward, *et al.* 2017 [26] Hawley, *et al.* 2016 [184]
	Prednisone, prednisolone and methylprednisolone	Corticoid therapy	Ward, *et al.* 2017 [26] Vogeser, *et al.* 2017 [185]
Estradiol	Estrone sulfate	Hormone replacement therapy (oral administration)	Thomas, *et al.* 1993 [186]
	Fulvestrant	Breast cancer therapy	Owen, *et al.* 2019 [11]
	Exemestane metabolites	Breast cancer therapy	Mandic, *et al.* 2017 [12]
Progesterone	Di-hydroprogesterone	Micronized progesterone therapy	Nahoul, *et al.* 1987 [187]
Testosterone	Fetal and placental steroids	Females, children	Taieb, *et al.* 2003 [188] Honour, 2010 [17]
Testosterone	DHEA-S	Females	Warner, *et al.* 2006 [189]
T3 (either total or free)	Triiodoacetic acid		Piketty, *et al.* 1996 [190]
DHEAS	Pregnenolone sulphate	Pregnancy	Krasowski, *et al.* 2014 [191]
Sandwich immunoassays			
ACTH 1-39	Fragments, precursor	Paraneoplastic tumors	Raff and Findling, 1989 [20]
Insulin	Pharmacological analogs	Inappropriate injection leading to hypoglycemia	Parfitt, *et al.* 2015 [22] Giurgea, *et al.* 2005 [24] Dayaldasani, *et al.* 2015 [23]
Growth hormone	Pegvisomant	Acromegaly therapy	Paisley, *et al.* 2007 [18] Manolopoulou, *et al.* 2012 [19]
	Placental GH	Pregnancy	Dias, *et al.* 2013 [192]

Abbreviations: ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone sulfate.

https://doi.org/10.3343/alm.2022.42.1.3

Clinical cases

Clinical case

Mr K (28/12/75)

- Malaria attack ➡ RF grade V and hypertension (200/120 mmHg)
- GFR = 6 mL/min et Cr= 4.7 g/L
- Proteinuria = 3044 mg/L ➡ 700 mg/g Creatinuria
- Asthenia and headaches

Clinical case

Mr K (28/12/75)

- Hypertension (200/120 mmHg)

→ Aldosterone quantification

RIA > 1000 ng/L

Clinical case

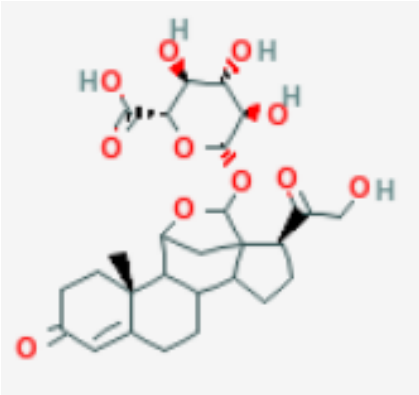
Mr K (28/12/75)

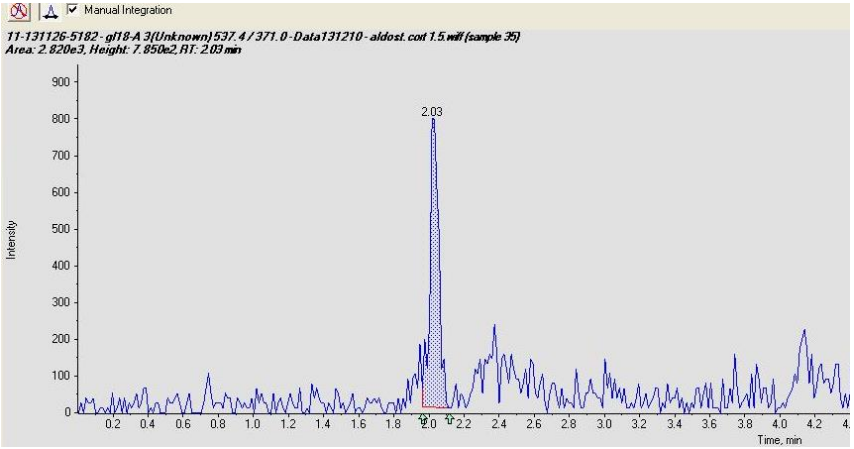
- Hypertension (200/120 mmHg)

→ Aldosterone quantification

RIA > 1000 ng/L vs LC-MS/MS = 66 ng/L (< 200 ng/L normal value)

➡ Presence of Aldosterone glucuronide





Clinical case

- 37-year-old female patient
- Invasive breast adenocarcinoma of no special type (NST)
- Left mastectomy
- Chemotherapy + radiotherapy + hormone therapy with chemical castration using Abemaciclib
- Salpingo-oophorectomy
- Biological monitoring of estradiol levels

- 49-year-old female patient
- Total hysterectomy with oophorectomy in 2021 for cervical cancer
- Breast cancer in 2023
  → Mastectomy
- Chemotherapy + radiotherapy + hormone therapy + Abemaciclib
- Biological monitoring of estradiol levels

Clinical case

Immunological method (Alinity®): 149 ng/L and 184 ng/L

Reference values:

- Premenopausal women: 21 – 312 ng/L
- Postmenopausal women without hormone replacement therapy: <28 ng/L

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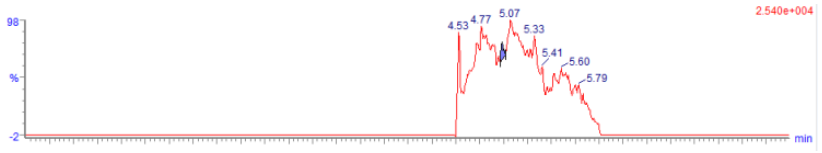
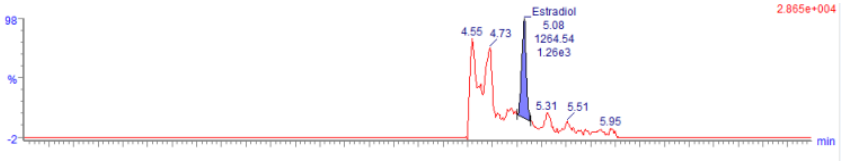
- Postmenopausal women without hormone replacement therapy: <28 ng/L

- LC-MS/MS method : < 5 ng/L



- Example of chromatogram in the presence of estradiol (~ 3 ng/L)".



Clinical case

- **What is the source of these interferences?**

- **Common treatment for both patients: Abemaciclib**
  Abemaciclib: a potent and selective inhibitor of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6)
  Treatment of hormone receptor (HR) positive, HER2 negative breast cancer with lymph node involvement and high risk of recurrence

➔ **Kit insert:**
"Samples from patients who have been administered treatments that inhibit tumor cell proliferation (e.g., CDK 4/6 inhibitors) may be subject to interference/cross-reactivity with the Alinity i estradiol assay."

➔recommended to measure estradiol by LC-MS/MS in this type of patient.

Clinical case

ED by GP :
- Hypertension
-Oedema **lower limbs and face**

MEDICAL HISTORY
•Pleural effusion at age 2
•Left testicular torsion

Men 23 yo

TREATMENTS
•Pantoprazole as needed

Examination

Asthenia

Muscle weakness

Oedema of lower limbs and face

Hypertension
(around 180 mmHg in systolic)

Clinical case

CTU 24h : 20692 µg/24H (3,5 – 45,0)
COR SAL midnight : 166,4 µg/L (<1,0)

METABOLISME PHOSPHO-CALCIQUE					
* Calcium	2.14		2.30	2.20-2.60	mmol/L
* Phosphates	1.00	non reçu	0.40	0.74-1.52	mmol/L
* PTH 3ème Génération (méthode Liaison)	43			4-33	ng/L
* 25-OH Vitamine D (méthode Liaison)	20			20-50	ng/mL
Consensus pour l'ensemble de la population belge selon le Belgian Bone Club					
ENDOCRINOLOGIE					
THYROIDE					
* TSH (méthode Alinity)	1.80			0.35-4.94	mUI/L
* T4 libre (méthode Alinity)	12.6			8.7-15.5	pmol/L
HYPOPHYSE ET GONADES					
ACTH (méthode Liaison)	?				ng/L
Analyse à faire directement					
STEROÏDES					
* Cortisol (méthode Alinity)	2113.8				nmol/L
Valeurs de référence : *- le matin : 102.1 - 535.2 nmol/L* *- l'après-midi : 80.0 - 477.3 nmol/L*					
HYPERTENSION					
Activité rénine couché (méthode LC-MS/MS)	0.10			0.20-1.40	ng/ml/h
CPRA (méthode LC-MS/MS)	0.10				ng/ml/h
* Aldostérone couché (méthode LC-MS/MS)	208			<75	ng/L
* Aldostérone/act. rénine couché	208.0			<20.0	
Résultat évocateur d'un hyperaldostéronisme primaire à confronter à l'aldostéronémie et à la clinique					

Clinical case

Thoraco-abdomino-pelvic CT scan

Large infiltrating and necrotic tumor lesion in the anterior-superior mediastinum.
Adjacent/confluent superior mediastinal lymphadenopathy.

- Germ cell tumor?
- Thymic tumor?
- Thymic lymphoma?
- Thymic neuroendocrine tumor?

Clinical case

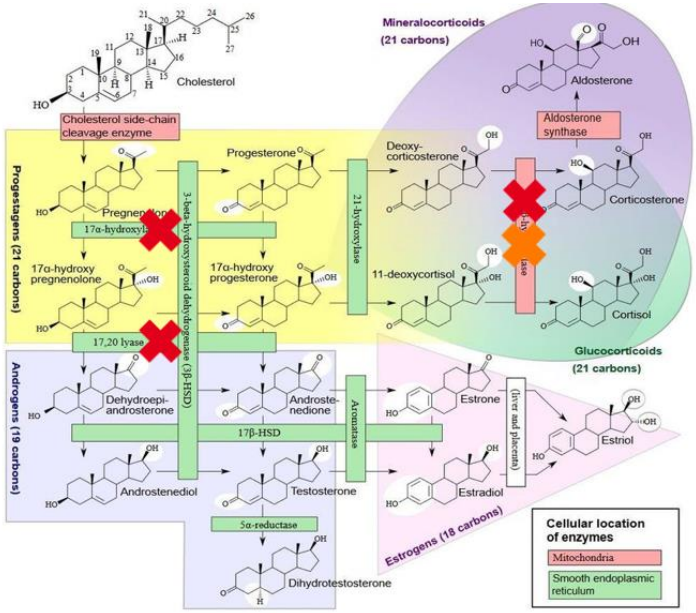
TREATMENTS

KETOCONAZOLE PO

Inhibitor of 11β-hydroxylase, 17α-hydroxylase, and 17,20-lyase.

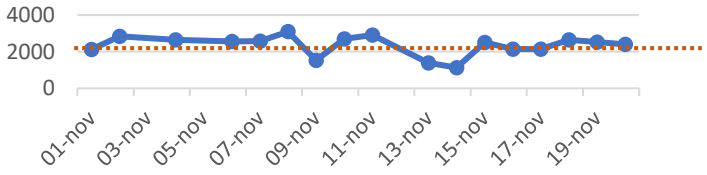
Treatment failure after 15 days and maximum dose reached.

➔ Initiation of IV Etomidate (3.1 mg/h).



KETOCONAZOLE
ETOMIDATE

Serum cortisol levels (Alinity) from arrival at the emergency department until discontinuation of ketoconazole



Clinical case

TREATMENTS

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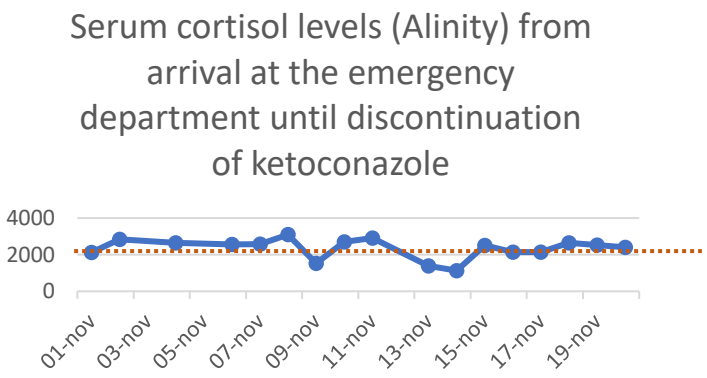
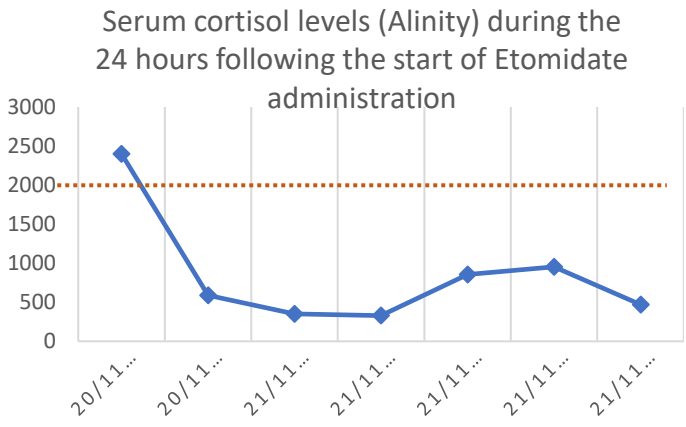
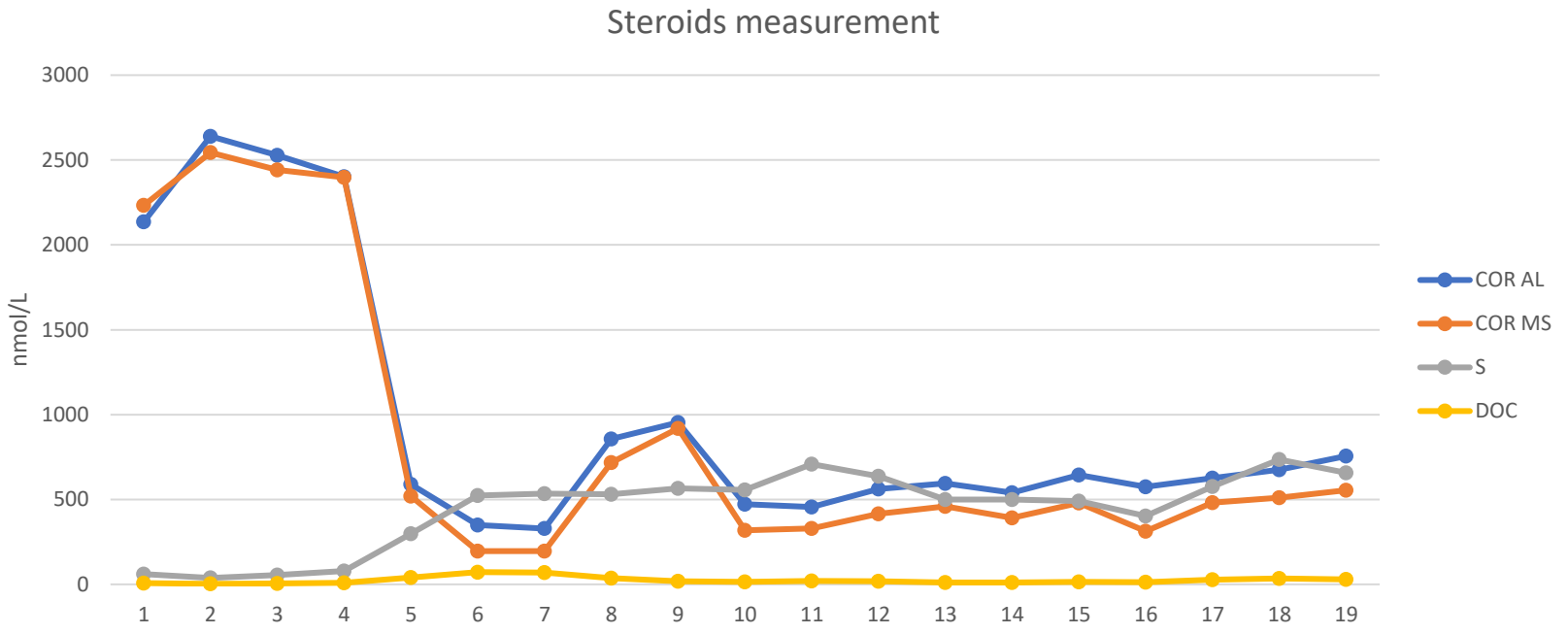


ETOMIDATE IV

Inhibitor of 11β-hydroxylase, with a dose-dependent effect.
The only available anti-cortisol treatment administered intravenously.
The effect is observed starting 5 hours after administration and reaches its maximum at 11 hours.



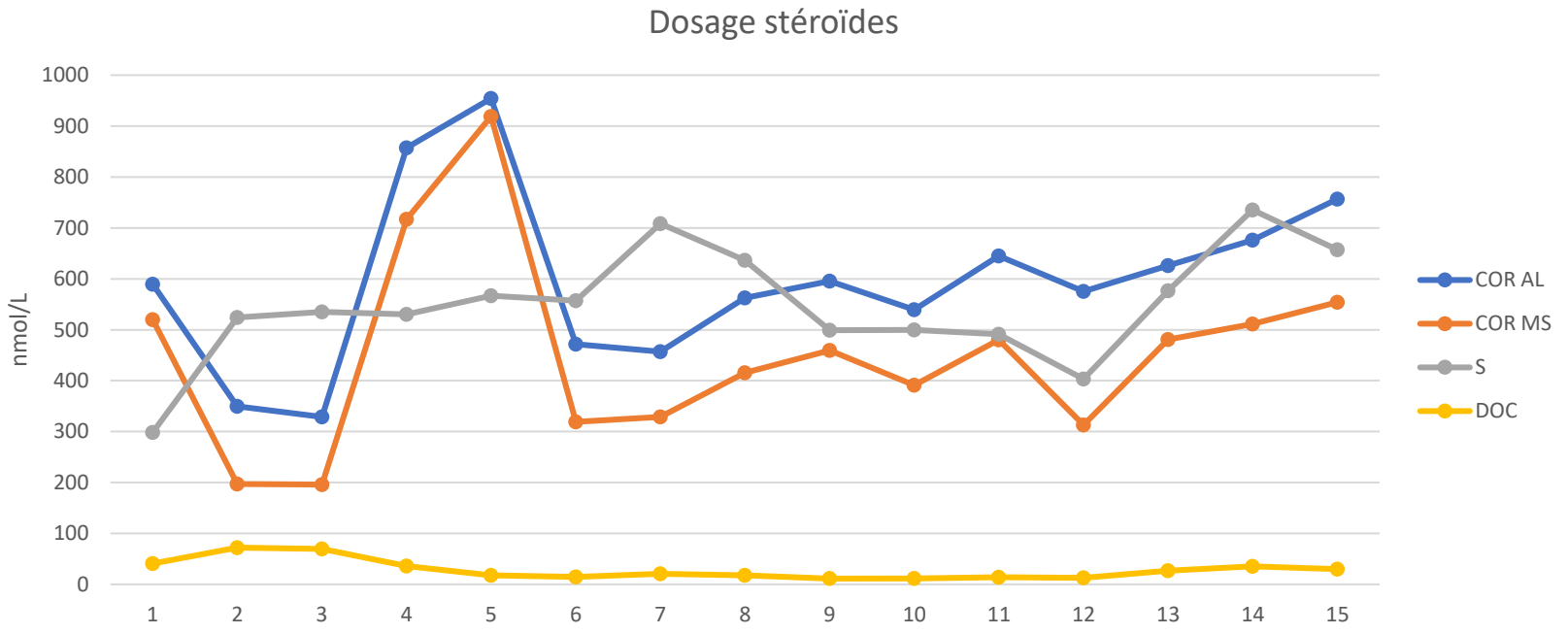
Clinical case



PATIENT SAMPLES

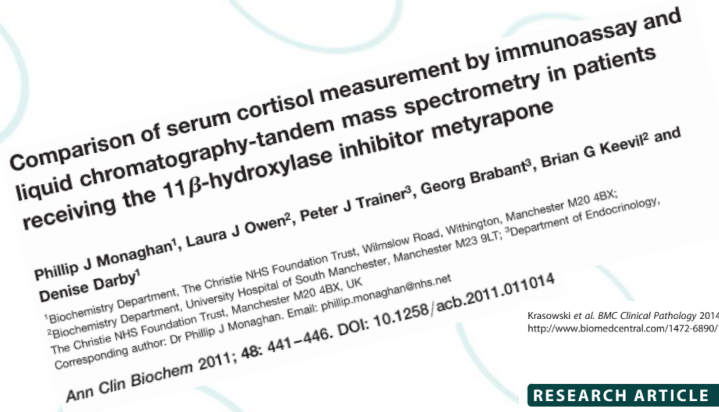
Average cross-reactivity of 26% with 11-deoxycortisol.

Clinical case



(zoom < 1000 nmol/L)

Clinical case



Comparison of serum cortisol measurement by immunoassay and liquid chromatography-tandem mass spectrometry in patients receiving the 11β-hydroxylase inhibitor metyrapone

Phillip J Monaghan[1], Laura J Owen[2], Peter J Trainer[3], Georg Brabant[3], Brian G Keevil[2] and Denise Darby[1]

[1]Biochemistry Department, The Christie NHS Foundation Trust, Wilmslow Road, Withington, Manchester M20 4BX; [2]Biochemistry Department, University Hospital of South Manchester, Manchester M23 9LT; [3]Department of Endocrinology, The Christie NHS Foundation Trust, Manchester M20 4BX, UK
Corresponding author: Dr Phillip J Monaghan. Email: phillip.monaghan@nhs.net

Ann Clin Biochem 2011; 48: 441–446. DOI: 10.1258/acb.2011.011014

Cortisol measurement in patients receiving metyrapone therapy

L J Owen[1], D J Halsall[2] and B G Keevil[1]

[1]Biochemistry Department, University Hospital of South Manchester, Manchester M23 9LT; [2]Clinical Biochemistry Department, Addenbrooke's Hospital, Cambridge, UK
Corresponding author: Laura Owen. Email: Laura.owen@uhsm.nhs.uk

Ann Clin Biochem 2010; **47**: 573–575. DOI: 10.1258/acb.2010.010167

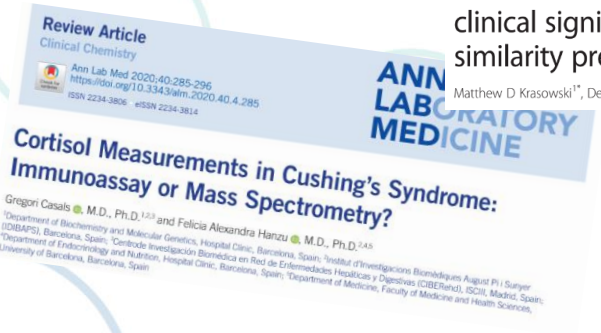
Krasowski *et al. BMC Clinical Pathology* 2014, **14**:33
http://www.biomedcentral.com/1472-6890/14/33

BMC Clinical Pathology

RESEARCH ARTICLE Open Access

Cross-reactivity of steroid hormone immunoassays: clinical significance and two-dimensional molecular similarity prediction

Matthew D Krasowski[1*], Denny Drees[1], Cory S Morris[1], Jon Maakestad[1], John L Blau[1,2] and Sean Ekins[3]



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ANNALS OF LABORATORY MEDICINE

Cortisol Measurements in Cushing's Syndrome: Immunoassay or Mass Spectrometry?

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Endocrinology, Diabetes & Metabolism CASE REPORTS

S W P Wong and others | Etomidate in Cushing's disease | ID: 19-0044; September 2019 DOI: 10.1530/EDM-19-0044

Etomidate in the management of severe Cushing's disease and MRSA bacteraemia in a district general hospital in the United Kingdom

Stephanie Wei Ping Wong[1], Yew Wen Yap[1], Ram Prakash Narayanan[1], Mohammad Al-Jubouri[2], Ashley Grossman[3], Christina Daousi[4] and Yahya Mahgoub[1]

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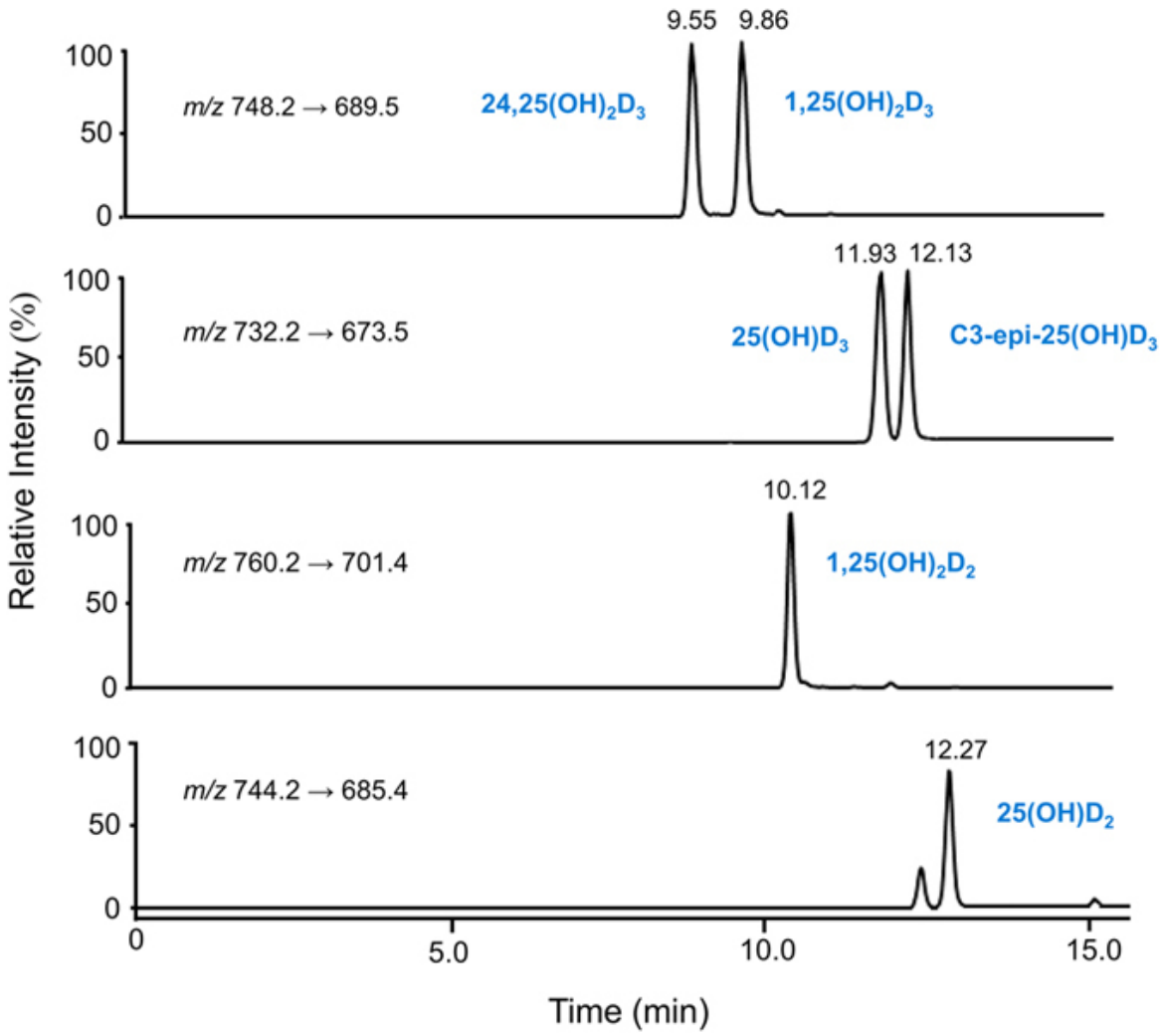
Clinical case

- 65-year-old male on chronic hemodialysis for end-stage renal disease (ESRD).
- Routine lab show undetectable 25(OH)D3 (<4 ng/mL) using an automated immunoassay (Alinity, Abbott).
- The patient reports adherence to vitamin D supplementation (cholecalciferol 1000 IU daily).
- Discrepancy between clinical picture (no signs of osteomalacia) and severe biochemical deficiency.
- Nephrologist questions assay reliability in dialysis context.
- Intervention:
  Sample reanalyzed by LC-MS/MS ➔25(OH)D3 = 22.5 ng/mL (sufficient).

Clinical case



Clinical case

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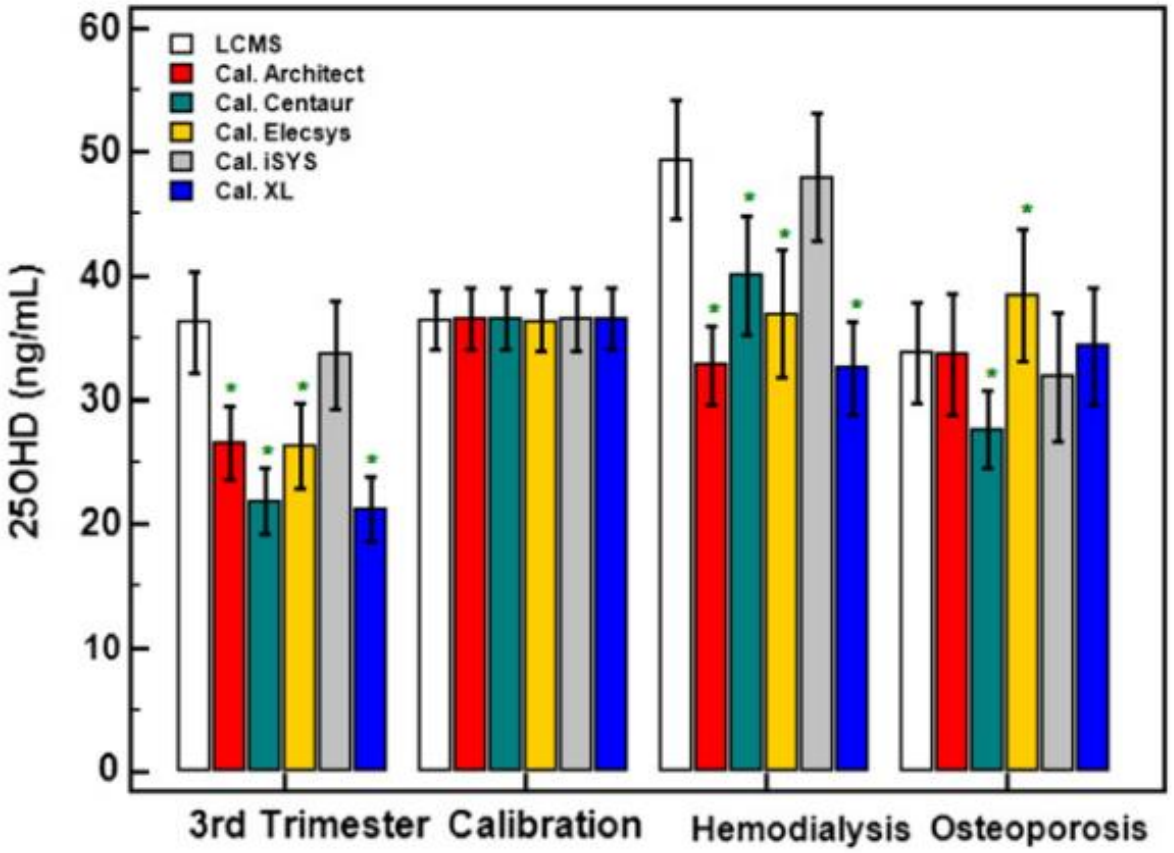
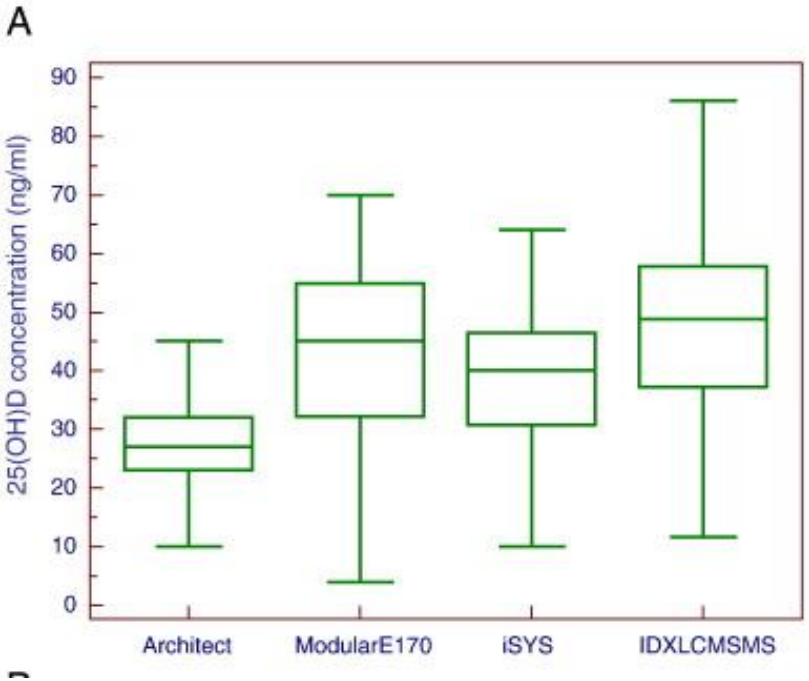


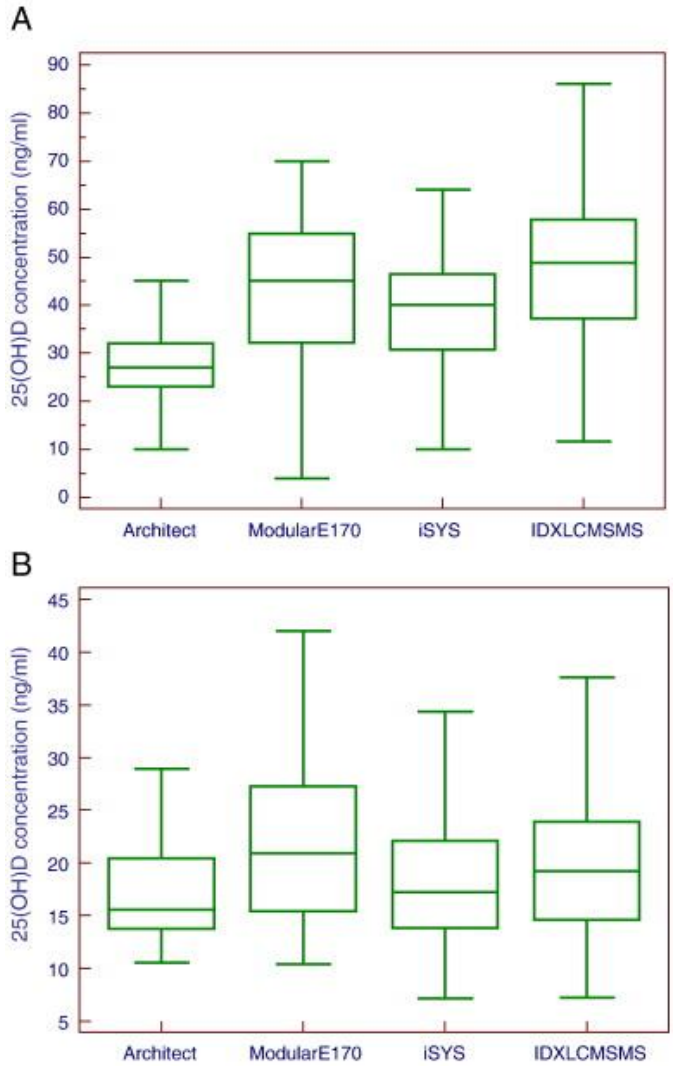
Clinical case



Box and Whisker plots showing distributions of 25(OH)D in 99 **hemodialysis patients** measured with Architect i2000sr (Abbott), Modular E170 (Roche), iSYS (IDS) and ID-XLC-MS/MS.

B. Depreter, A.C. Heijboer, M.R. Langlois, Accuracy of three automated 25-hydroxyvitamin D assays in hemodialysis patients, Clin Chim Acta, 415 (2013), pp. 255-260

Clinical case



Box and Whisker plots showing distributions of 25(OH)D in 99 **hemodialysis patients** (A) and 50 healthy subjects (B) measured with Architect i2000sr (Abbott), Modular E170 (Roche), iSYS (IDS) and ID-XLC-MS/MS.

B. Depreter, A.C. Heijboer, M.R. Langlois, Accuracy of three automated 25-hydroxyvitamin D assays in hemodialysis patients, Clin Chim Acta, 415 (2013), pp. 255-260

Clinical case

Table2. Numbers of patients classified according to vitamin D status with the measured 25(OH)D concentrations (ng/ml) by each method in hemodialysis patients and healthy subjects.

Assay and sample group	Deficient <10ng/ml	Insufficient <20ng/ml	Sufficient 20–30ng/ml	Optimal >30ng/ml
Hemodialysis patients (n=99)				
Architect (Abbott)	0	15	48	36
Modular E170(Roche)	2	5	15	77
iSYS (IDS)	0	2	18	79
ID-XLC-MS/MS	0	3	12	84
Healthy subjects (n=50)				
Architect (Abbott)	0	33	14	3
Modular E170(Roche)	0	23	16	11
iSYS (IDS)	5	22	17	6
ID-XLC-MS/MS	2	24	18	6

Clinical case

Patient:
65-year-old male on chronic hemodialysis for end-stage renal disease (ESRD).

Presentation:
Routine lab show **undetectable 25(OH)D (<4 ng/mL)** using an **automated immunoassay (Alinity, Abbott)**.

The patient reports adherence to vitamin D supplementation (cholecalciferol 1000 IU daily).

Clinical Concern:
Discrepancy between **clinical picture (no signs of osteomalacia)** and **severe biochemical deficiency**.

Nephrologist questions assay reliability in dialysis context.

- **Intervention:**
  Sample reanalyzed by **LC-MS/MS** ➔**25(OH)D_3 = 22.5 ng/mL** (sufficient).

Explanation:

- Immunoassay failed due to **incomplete dissociation of 25(OH)D from DBP**, aggravated by **elevated urea and matrix effects**.
- LC-MS/MS bypassed this by **organic protein precipitation** and **specific detection**.

IA versus LC-MS/MS

Criteria	*Immunoassays (IA/RIA)*	*Mass Spectrometry (MS)*
Principle	Uses antibodies to detect specific steroids	Separates and detects molecules based on mass-to-charge ratio
Sensitivity	Good	Excellent
Specificity	**Moderate to low** (cross-reactivity is common)	**High** (distinguishes structurally similar compounds)
Interferences	Frequent (e.g., heterophilic antibodies, rheumatoid factor)	Rare (if sample prep is adequate)
Precision / Accuracy	Variable; depends on antibody quality	Very high
Multiplexing capability	Limited (typically one analyte per assay)	Yes (can measure multiple steroids simultaneously)
Cost & Accessibility	Lower cost; widely used in routine labs	Higher cost; increasingly available
Typical errors	**Underestimation or overestimation** due to interferences	Fewer errors; requires technical expertise

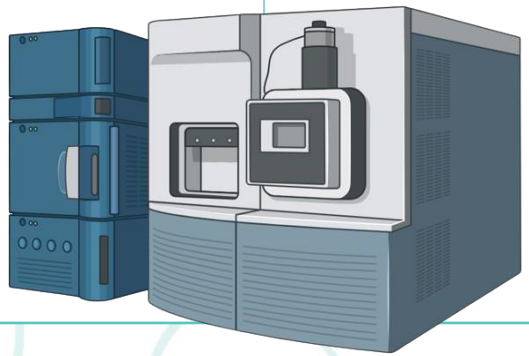
LC-MS/MS

Advantages

- High sensivity
- Specificity
- Reproducibility
- Ability to perform simultaneous analysis
- No variation LOT to LOT

Disadvantages

- Price
- Method development
- Technical aspect
- Noise and heat generation
- Nitrogen generator and compressed air or linked supply
- Review of peaks one by one
- Annual maintenance contract



Acknowledgments

My team: “Department of clinical chemistry in the University of Liege. Belgium”

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