

ERNDiM

Quality Assurance in Laboratory Testing for IEM

Special Assays in Serum (SAS) technical meeting

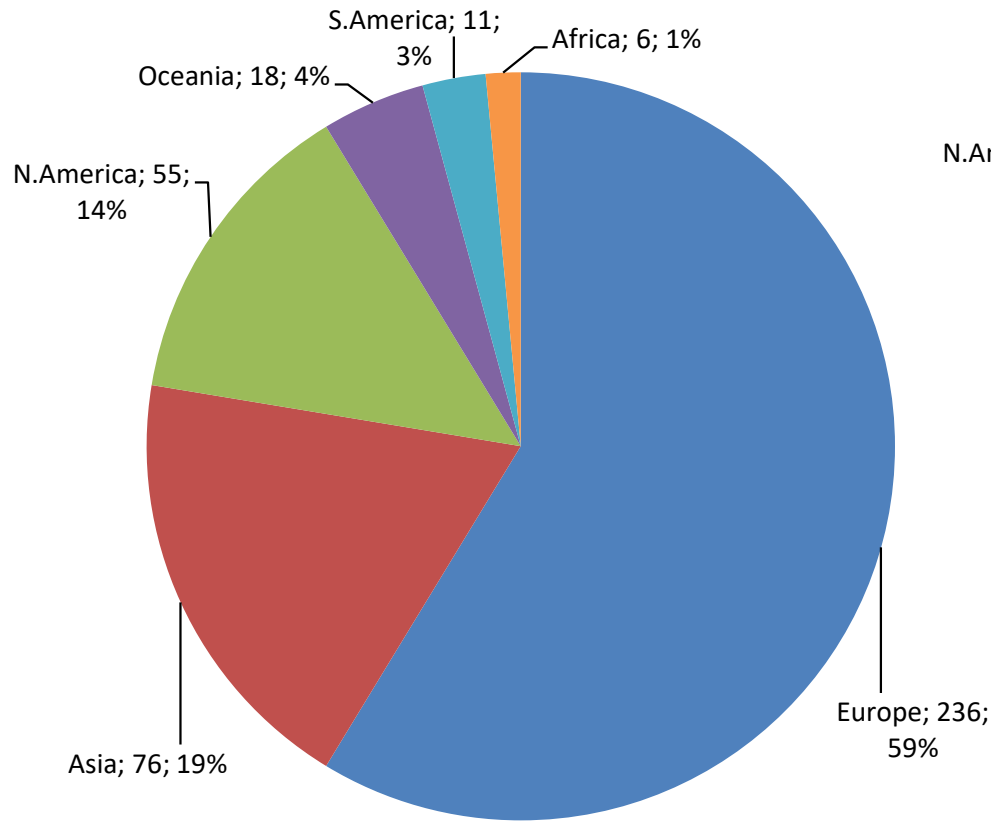
Rafa Artuch, Scientific Advisor

09/04/2026

Question: Highlighting metabolites with the greatest variability among laboratories of SAS and SAU.

Participants

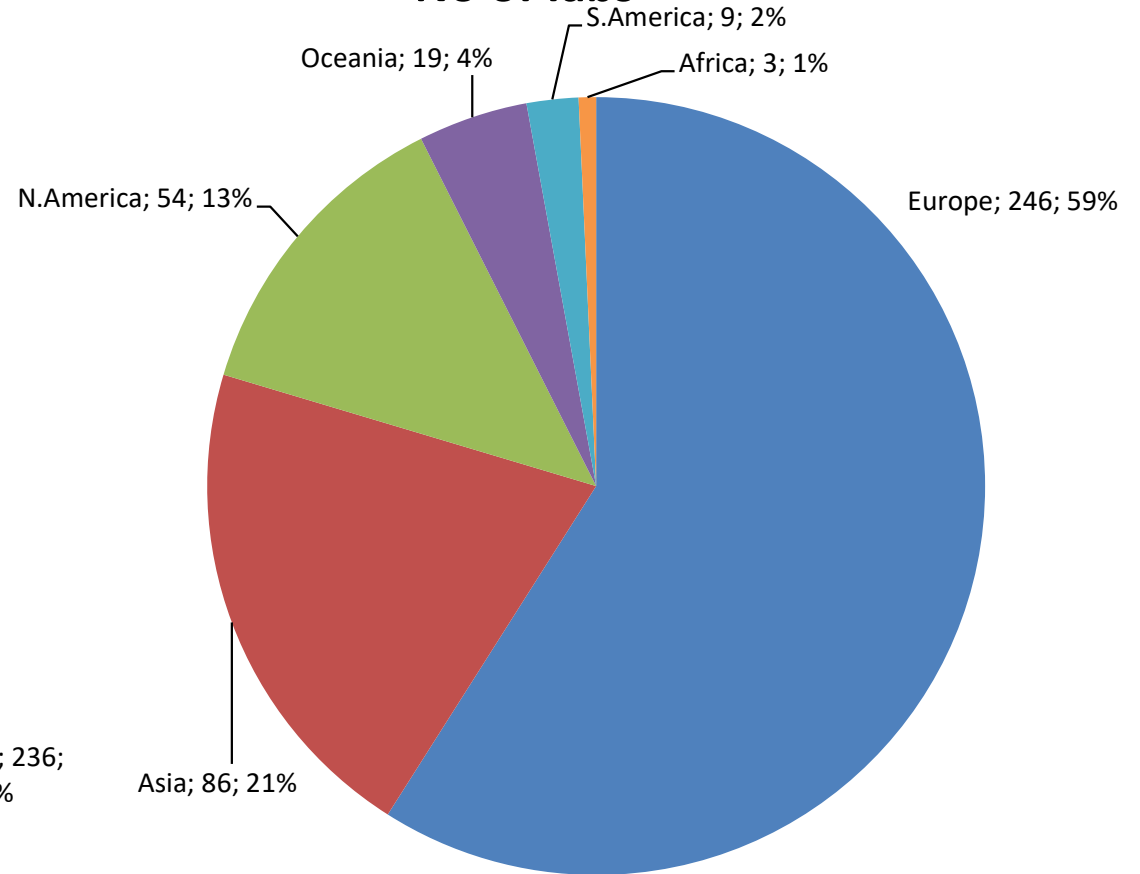
No. of labs



2020: Number of participants = 402

- Number of participating countries = 62

No of labs



**2025: Number of participants = 417
(3.7% ↑ on 2020)**

- Number of participating countries = 61

EQA Scheme:	Special Assays serum SAS
Total. No. of datasets/labs: <i>(please delete as appropriate)</i>	254
Any general comments or problems?	<p>19/25 below 20% Interlab CV 4/25 between 20-30%. CoQ has improved a lot (28%). The worst Lysosphingomyelin (32%) and succinylacetone (43%). Galactose and pyruvate issues seems solved with the antibiotics added to the matrix.</p>
Any changes proposed for next scheme year?	<p>Movement of some metabolites to LIS.</p> <p>To include GDF-15 and FGF-21 as pilot metabolites (residual amounts in the matrix).</p>

Analyte	Units	Added Amounts			
		Sample Pair 2025. 01 - 08	Sample Pair 2025. 02 - 05	Sample Pair 2025. 03 - 07	Sample Pair 2025. 04 - 06
3-OH-Butyric Acid	mmol/L	2.44	0.94	0.00	3.94
Biotinidase*	nmol/min/ml serum				
C22:0 Behenic acid	µmol/L	55	15.9	0	35
C24:0 Lignoceric acid	µmol/L	46	30	1.06	11.0
C26:0 Cerotic acid	µmol/L	1.04	9.13	0.00	4.14
C26:0 LPC	µmol/L	1.46	0.78	0.14	0.30
Carnitine Free	µmol/L	102	62	0	11.9
Creatine	µmol/L	42	23	0	63
Galactose	µmol/L	1199	1899	50	500.
Guanidino acetic acid	µmol/L	12.9	6.4	1.2	19.1
Homocysteine	µmol/L	200	40	00	15.1
Lactic Acid	mmol/L	2.19	6.49	0	4.19
Methylmalonic acid	µmol/L	400	50	0	2.14
NEFA*	µmol/L				
Phytanic acid	µmol/L	7.03	22	0.00	15
Pipecolic Acid	µmol/L	20	8.3	0.00	38
Pristanic acid	µmol/L	7.15	4.96	0.00	1.97
Pyruvic Acid	mmol/L	0.20	0.15	0.05	0.10
Succinylacetone	µmol/L	4.06	1.64	0.00	0.81

Interlab CV < 10%

Analyte	Units	Added Amounts			
		Sample Pair 2025. 01 - 08	Sample Pair 2025. 02 - 05	Sample Pair 2025. 03 - 07	Sample Pair 2025. 04 - 06
7-Dehydrocholesterol	µmol/L	73	168	0	9.02
7-Ketocholesterol	µmol/L	0.50	1.50	0.10	1.00
Cholestane-3b. 5a. 6b-triol	µmol/L	0.80	0.50	0.05	0.30
Cholestanol	µmol/L	13.02	93	0	68
Cholesterol*	mmol/L				
Coenzyme Q10	µmol/L	4.02	2.01	0	1.0
Glucosylsphingosine	nmol/L	599	402	0	1002
Lyso Gb3	nmol/L	99	50	0	9.8
Lysosphingomyelin	nmol/L	400	39	0	20

Cytokines

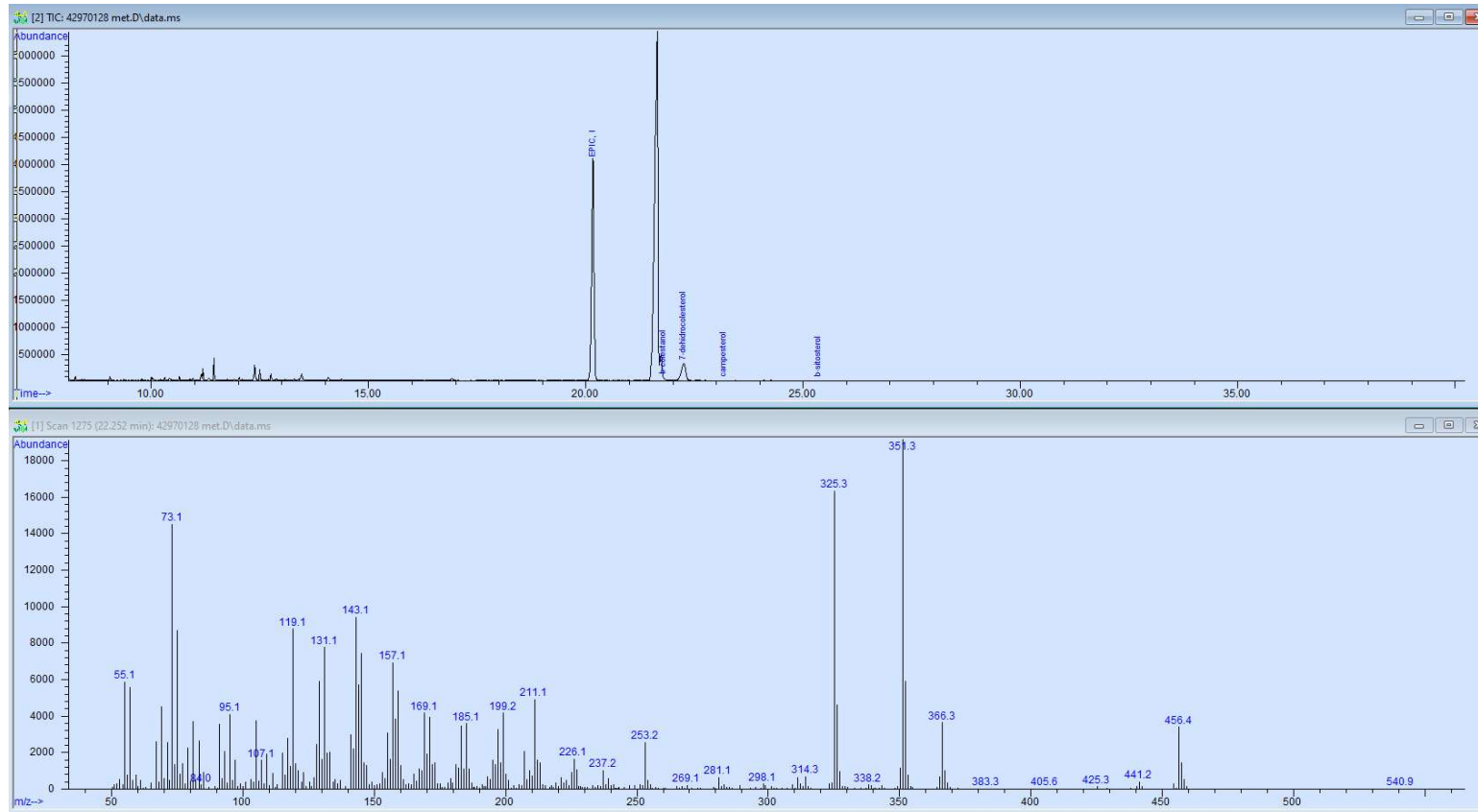
- **GDF-15** → In mitochondrial translation and deletion/mutation defects with muscle and other tissues involvement. But increased in other conditions (cachexia, inflammation, cardiac diseases) → *Sensitive/less specific*
- **FGF-21** → mtDNA translation defects and deletions with muscle involvement. Regulates lipid and glucose metabolism (Obese, metabolic syndrome). From skeletal muscle and heart → *Specific/less sensitive*

Circulating GDF15 and FGF21 are reliable biomarkers for children with PMDs. GDF15 can serve as a screening biomarker for diagnosis, and FGF21 can serve as a severity biomarker for monitoring, but.....

Harmonization of GDF-15 and FGF-21 measurements

Automated vs ELISA methods

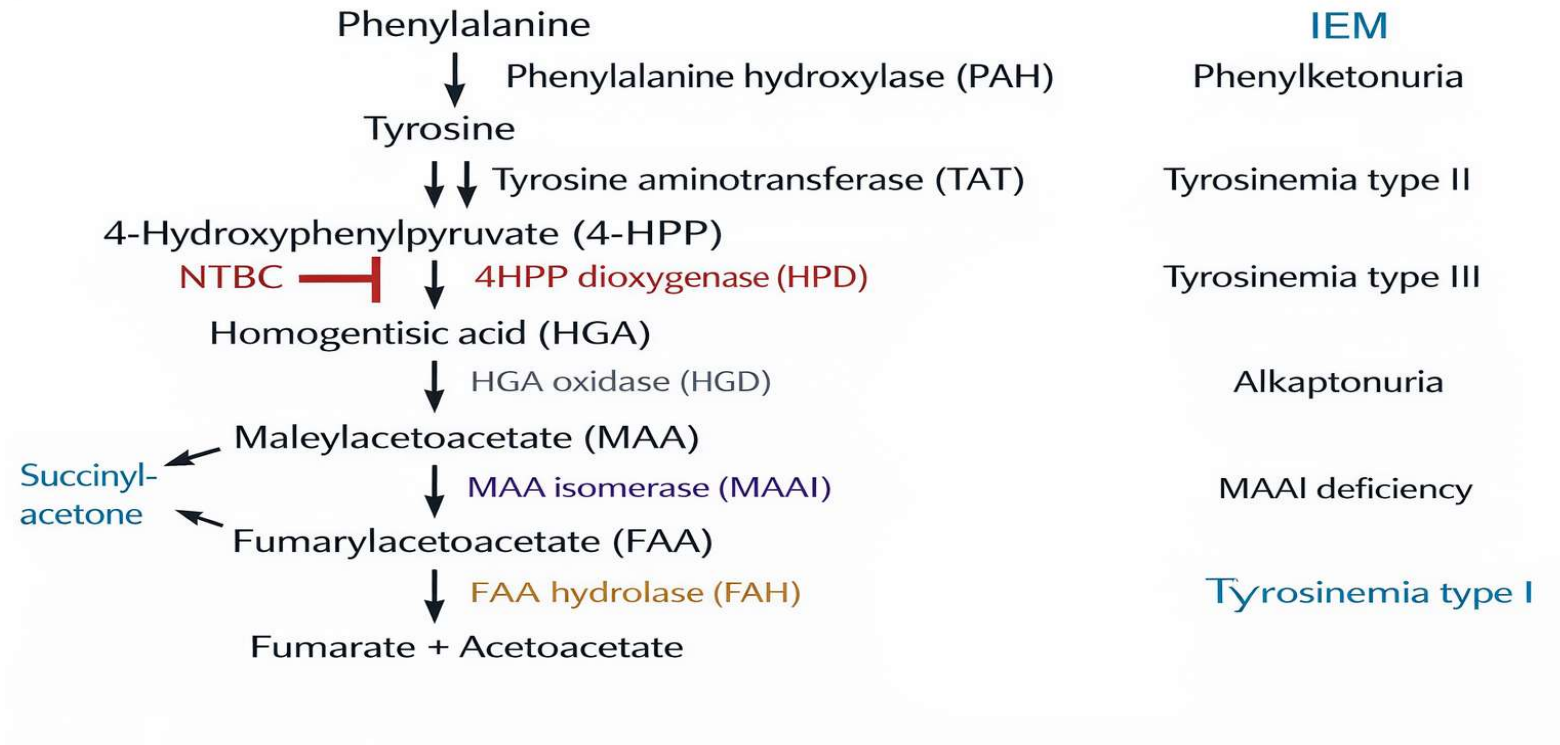
Analyte	CV interlab (%) SD/Mean*100	Added Amounts			
		Sample Pair 2025. 01 - 08	Sample Pair 2025. 02 - 05	Sample Pair 2025. 03 - 07	Sample Pair 2025. 04 - 06
7-Dehydrocholesterol	28.3	73	168	0	9.02
Coenzyme Q10	28.8	4.02	2.01	0	1.0
Glucosylsphingosine	25.7	599	402	0	1002
Lyso Gb3	27.3	99	50	0	9.80
Lysosphingomyelin	32	400	39.9	0	20.3
Succinylacetone	43	4.06	1.64	0.00	0.81



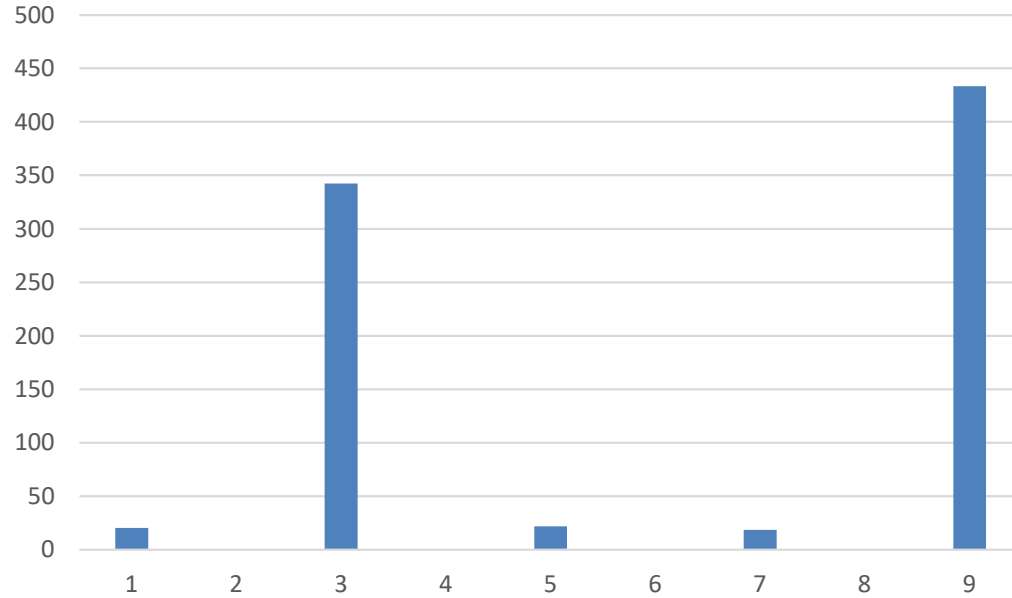
Consider psychiatric treatments that inhibit DHCR7: haloperidol, aripiprazole, cariprazine, fluoxetine, trazodone and amiodarone.

Succinylacetone ($\mu\text{mol/L}$)

a



Succinylacetone (µmol/L)



Mean = 3.2 Mean = 0.03 Mean = 0.61 Mean = 1.35 Mean = 0.06
 SD = 0.65 SD = 0.089 SD = 0.13 SD = 0.25 SD = 0.26
 %CV = 20 %CV = 342 %CV = 22 %CV = 19 %CV = 433
Target = 4 **Target = 0** **Target = 0.81** **Target = 1.64** **Target = 0**

(n=17). 12 LC-MS, 4 GC/MS, 1 other

Scale Standard Deviations	Scale µmol/L
>3SD	>2.11
2-3SD	1.86 - 2.11
1.5 - 2.0SD	1.73 - 1.86
1.0 - 1.5SD	1.61 - 1.73
0.5 - 1.0SD	1.48 - 1.61
0.0 - 0.5SD	1.35 - 1.48
-0.5 - 0.0SD	1.23 - 1.35
-1.0 - -0.5SD	1.10 - 1.23
-1.5 - -1.0SD	0.977 - 1.10
-2 - -1.5SD	0.851 - 0.977
-3 - -2SD	0.599 - 0.851
<-3SD	<0.599

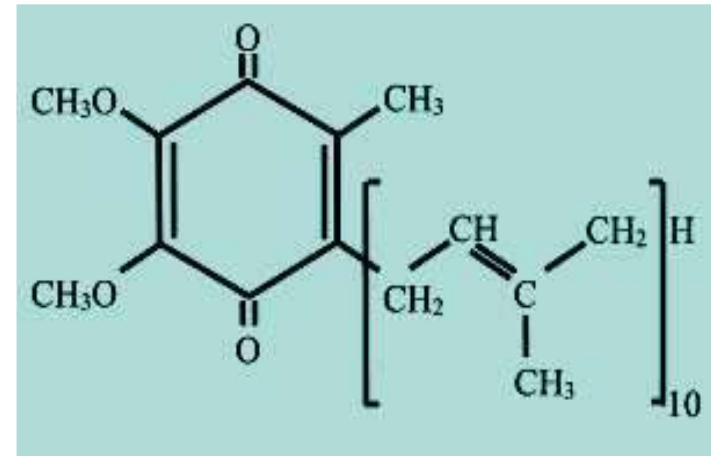
Enzymatic - spectrophotometric	LC-MS/MS
GC-MS - TMS - LLE - oximation	Other
GC-MS - TMS - LLE - oximation - SID	Your Lab
GC-MS - TMS - LLE - SID	



Coenzyme Q₁₀

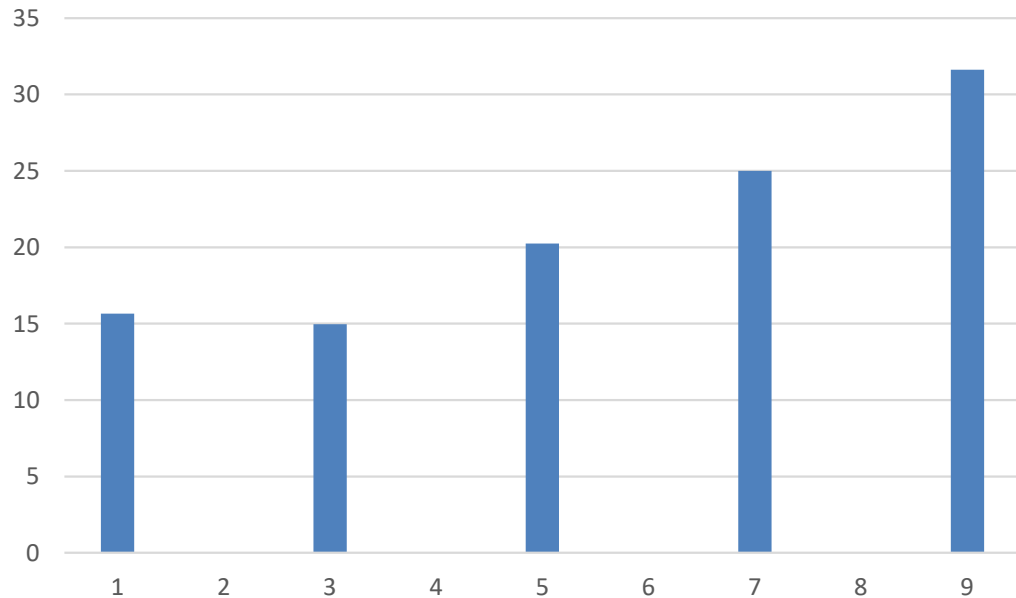
- **Respiration** (Crane *et al.* 1956).
- **Antioxidant** (Witting *et al.* 2000).
- **Plasma membrane** (Villalba *et al.* 1995).
- **Uncoupling proteins** (Echtay *et al.* 2000).
- **Apoptosis** (Fontaine *et al.* 1998).
- **Pyrimidine biosynthesis** (López-Martín *et al.* 2007).
- **β-oxidation (FFA)** (Gempel *et al.* 2007).

- Plasma, PBMC, platelets, urine, muscle, fibroblasts...



Primary deficiency → no detected in plasma, only in muscle, fibroblasts.
Secondary deficiencies of CoQ are common among PMM
High variability among laboratories (ERNDiM)
Plasma is a good surrogate for treatment monitoring

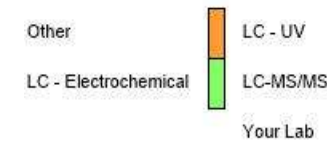
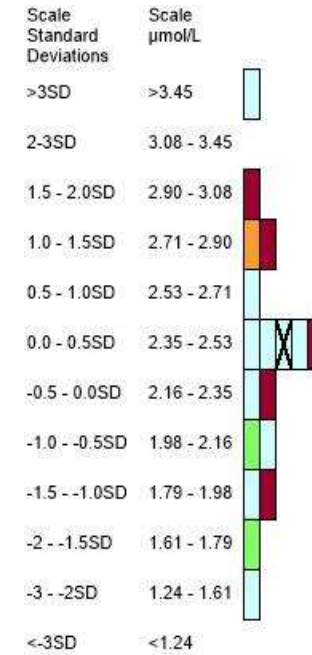
Coenzyme Q10 ($\mu\text{mol/L}$)



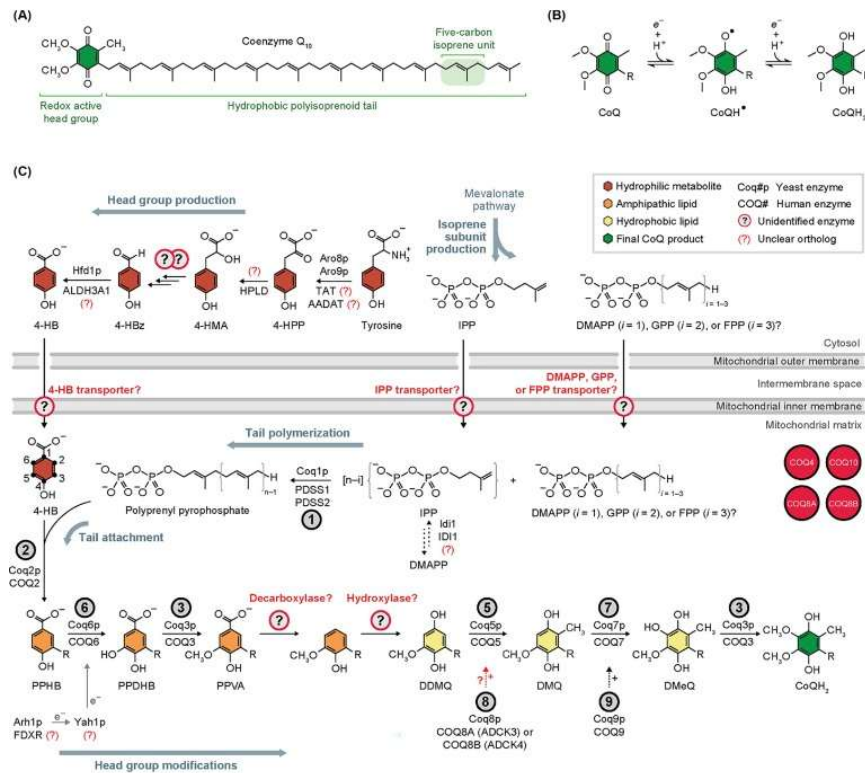
Mean = 2.35	Mean = 0.54	Mean = 1.17	Mean = 1.54	Mean = 3.1
SD = 0,36	SD = 0.081	SD = 0.24	SD = 0.38	SD = 0.98
%CV = 16	%CV = 15	%CV = 20	%CV = 25	%CV = 32
Target = 4	Target = 0	Target = 1	Target = 2	Target = 4

(n=18). 10 LC-MS/MS, 5 LC-UV, 1 LC-ED, 1 others

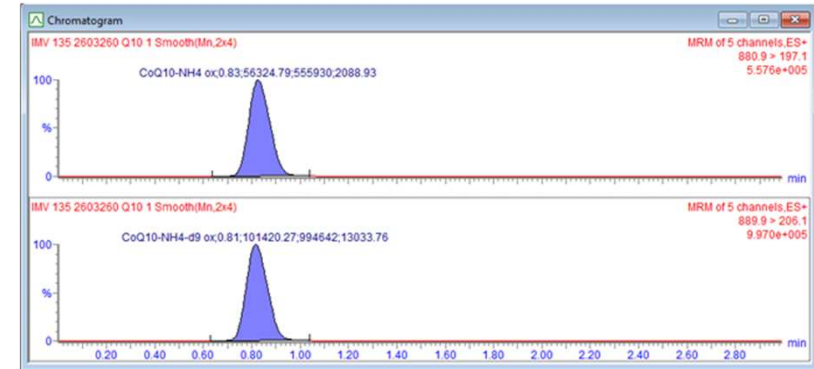
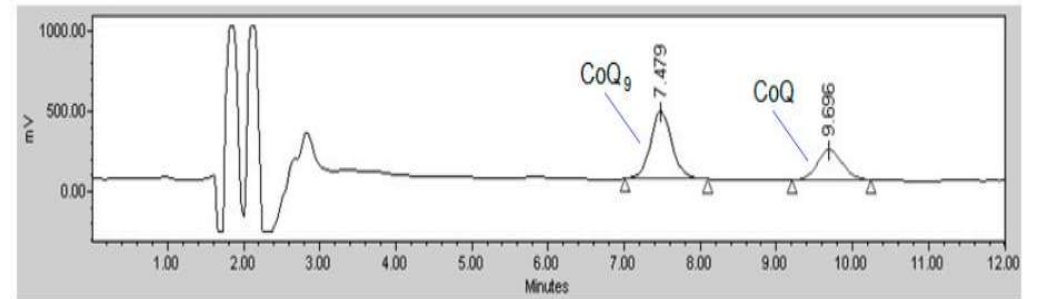
Pairs 1-8: 3.1 \rightarrow 2.38 (real target 4.5)
 Pairs 2-5: 1.74 \rightarrow 1.54 (real target 2.5)
 Pairs 3-7: 0.54 \rightarrow 0.55 (real target 0.55)
 Pairs 4-6: 1.1 \rightarrow 1.1 (real target 1.5)



Coenzyme Q₁₀



Trends in Biochemical Sciences



Question: The CoQ10 variability and normal reference ranges

Serum/Plasma

0.4-1.1 μmol/L. Cholesterol dependent

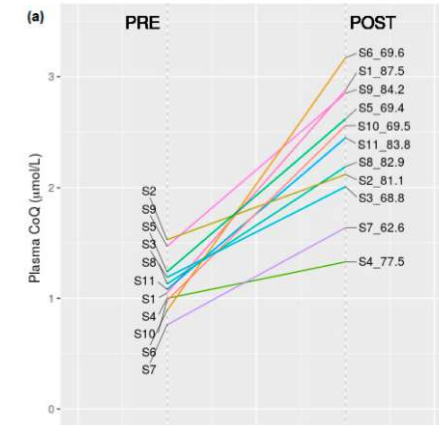
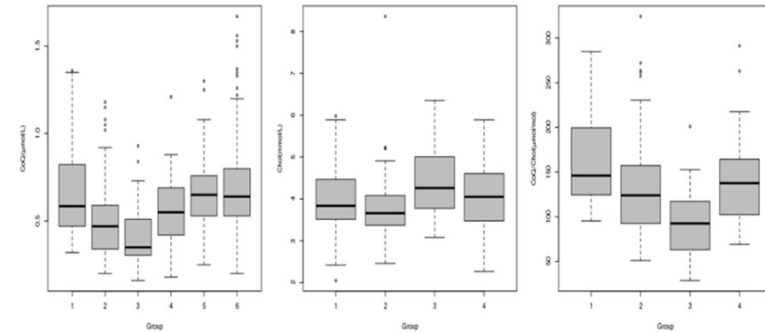
Low values: PKU, lysosomal storage diseases, hypocholesterolemias

Up to 20% nutritional sources

Preanalytical issues (instability)

Isomer (trans) due to light

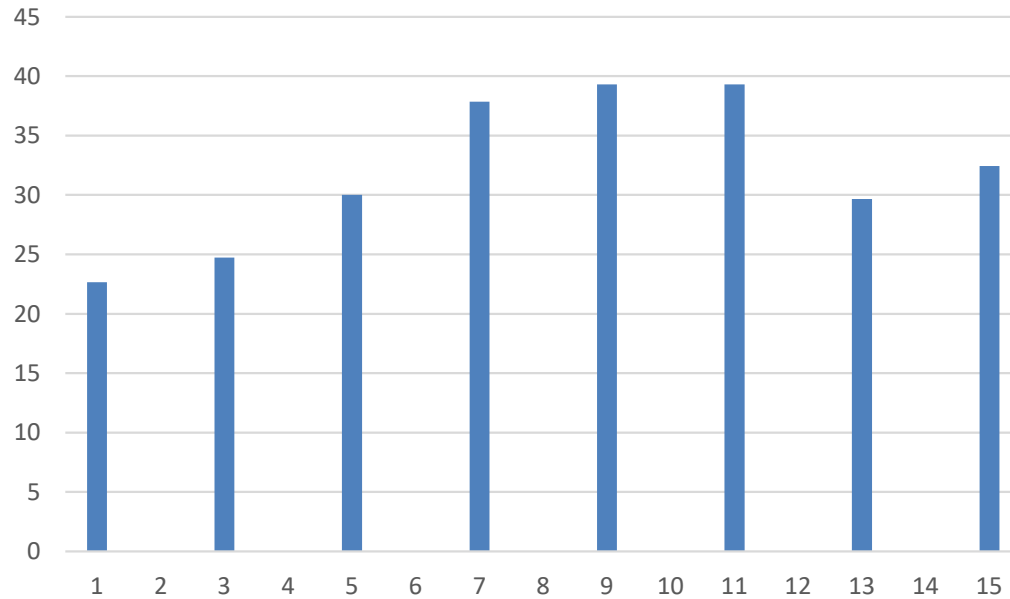
Both reduced and oxidized forms.



	2020 (n = 11)	2021 (n = 13)
Mean values detected (μmol/L)	0.757	0.901
Precision (CV of the duplicates, %)	52.7	50.5
Linearity (r)	0.782	0.664
Recovery (% of added CoQ)	27	26
Interlaboratory CV (%)	141	82.2

2025 (n=18) → 28.8%

Lysoesphingomyelin ($\mu\text{mol/L}$)



Mean = 300	Mean = 7.2	Mean = 20	Mean = 37
SD = 102	SD = 1,72	SD = 6	SD = 14
%CV = 23	%CV = 25	%CV = 30	%CV = 38
Target = 400	Target = 0	Target = 20	Target = 40

Scale Standard Deviations	Scale nmol/L	
>3SD	>72.5	
2-3SD	60.5 - 72.5	
1.5 - 2.0SD	54.6 - 60.5	
1.0 - 1.5SD	48.6 - 54.6	
0.5 - 1.0SD	42.6 - 48.6	
0.0 - 0.5SD	36.7 - 42.6	
-0.5 - 0.0SD	30.7 - 36.7	
-1.0 - -0.5SD	24.7 - 30.7	
-1.5 - -1.0SD	18.8 - 24.7	
-2 - -1.5SD	12.8 - 18.8	
-3 - -2SD	0.862 - 12.8	
<-3SD	<0.862	

Target 40

(n=26). LC-MS.

LC-MS/MS Your Lab

Recovery

Phytanic = 51% (low recovery only at high concentrations: 15 and 22 umol/L)

Precision

Coenzyme Q10 = 21%

Lysosphingomyelin = 21%

Questions

- The increase of pipercolic acid in plasma and/or urine?
 - www.iembase.org: Antiquitin deficiency (↑↑), peroxisomal disorders (PEX1,2,3,5,6,10,11B,12,13,14,16,19,26, ACOX1, D-bifunctional protein, classic Refsum), and Mitochondrial oxodicarboxylate carrier deficiency. Isolated sulfite oxidase deficiency and MOCS1, 2 and Gephryn (↑) in CSF
 - Dietary increments: No relevant (vegetables).
 - Caution in neonates (can be normal).
 - Alternatives: Alpha-AASA, P6C and 6-Oxo-Pip.

- Is it possible to include total carnitine concentration in the SAS scheme?

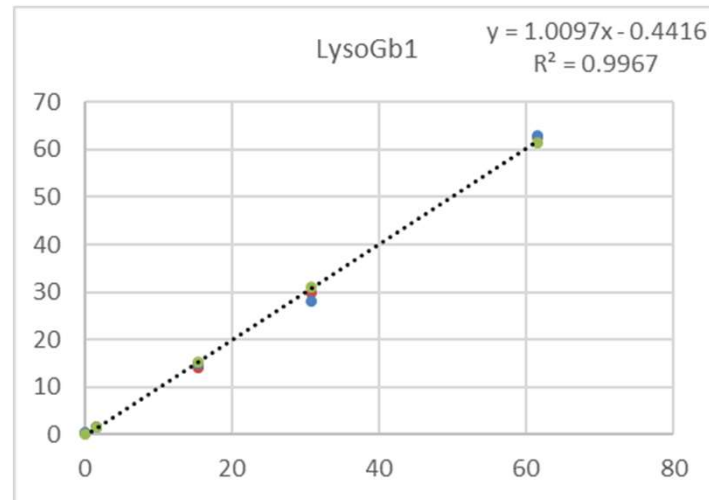
It was done in the past, but with not very good results. Moreover, probably it is too expensive, since certain acylcarnitine species would need to be added. On the other hand, schemes for the acylcarnitine profile already exist. Finally, the use of total carnitine for diagnosis is limited.

- Lysosomal markers (Lyso-GB1: Gaucher) - relevance of concentrations - construction of calibration curves (solubility issues).

5mg of the Internal or external standard in 10 ml chloroform:methanol (2:1)

Linearity: 0-61 nmol/L

Ref Val = 0.21-1.8 nmol/L



LIS 2026:

Esterols: Cholestanol, Cholesterol, Coenzyme Q10, Desmosterol, 7-dehydrocholesterol, Sitosterol and Lathosterol

Others: C26:0-lysophosphatidylcholine (C26:0-lysoPC), Glucosylsphingosine, Lyso-globotriaosylceramide (Lyso-Gb3), Lyso-monosialoganglioside 1 (Lyso-GM1), Lyso-monosialoganglioside 2 (Lyso-GM2), Lyso-sphingomyelin (Lyso-SM), N-palmitoyl-O-phosphocholineserine, (PPCS).

ERNDiM

Quality Assurance in Laboratory Testing for IEM

THANKS

Remember the annual survey for adding new ideas.