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Quality Assurance in Laboratory Testing for IEM

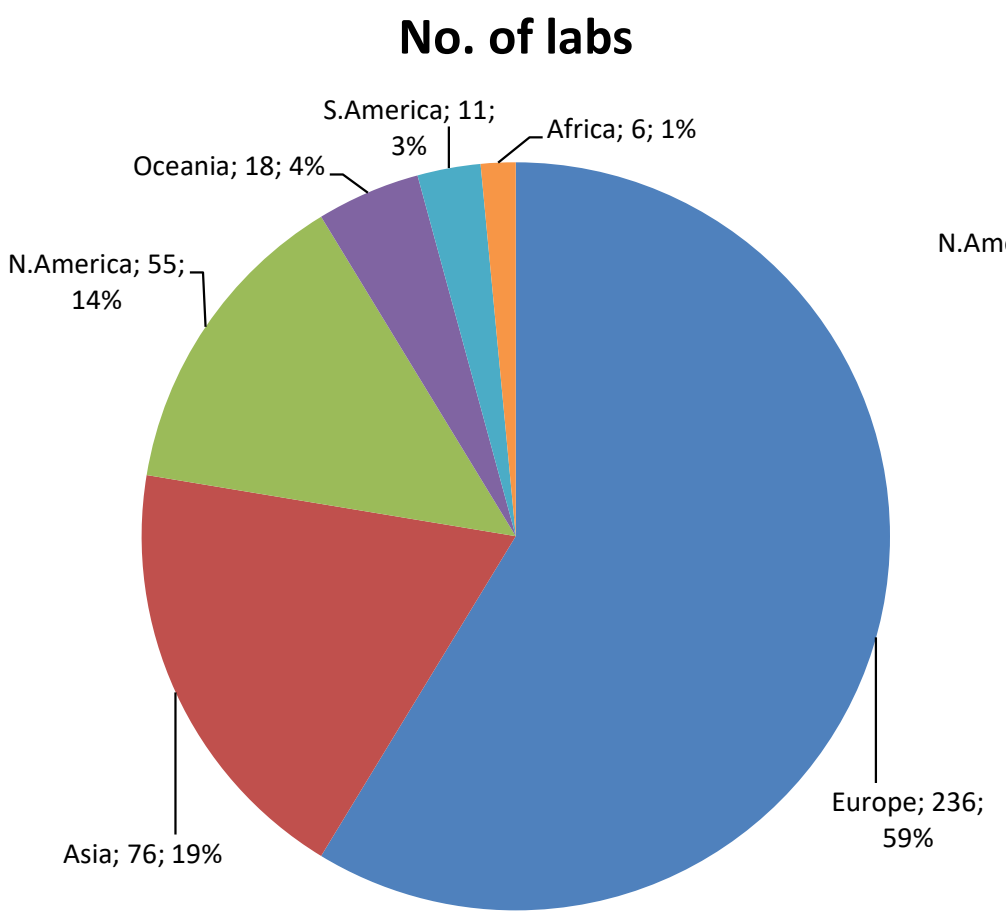
Special Assays in Urine (SAU) technical meeting

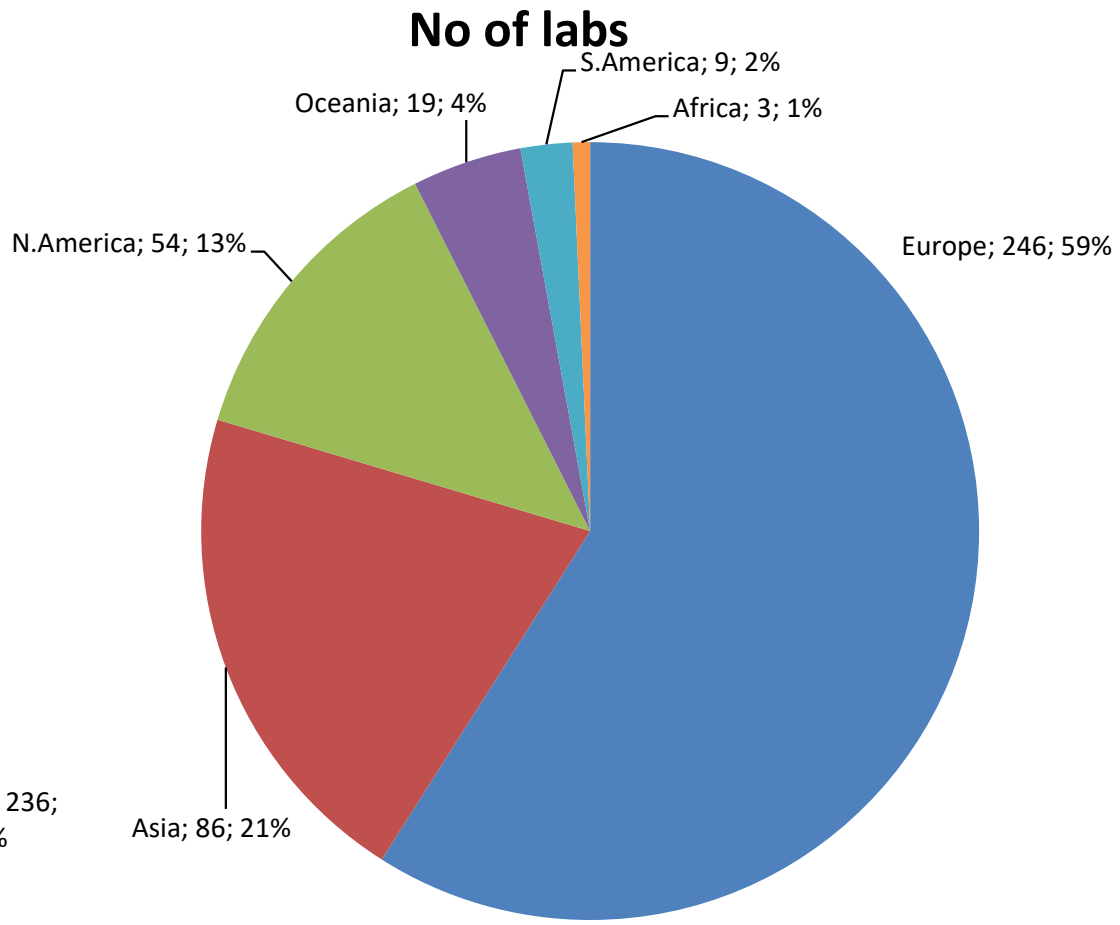
Rafa Artuch, Scientific Advisor

09/04/2026

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

Participants





2020: Number of participants = 402

- Number of participating countries = 62

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

- Number of participating countries = 61

EQA Scheme:	**Special Assays urine SAU**
Total. No. of datasets/labs: *(please delete as appropriate)*	195
Any general comments or problems?	Interlab CV below 20% in 14/22 Between 20-30% in 4/22 The worst (CV > 40%) for 4-OH-Glutamic, D.L-glyceric, Aminolevulinic and oxalic acids, and succinylacetone.
Any changes proposed for next scheme year?	**To add phosphoethanolamine for hypophosphatasia.**

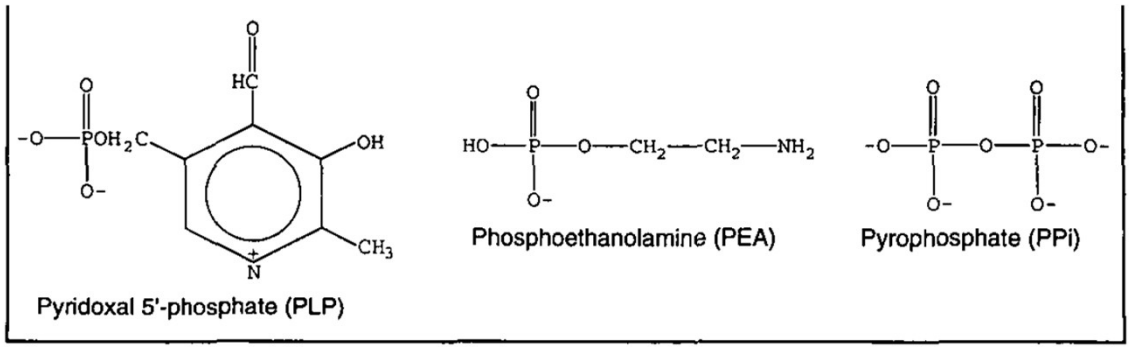


Fig 1. TNSALP substrates: pyridoxal-5'-phosphate, phosphoethanolamine, and pyrophosphate.

https://www.aapd.org/globalassets/media/publications/archives/hu-18-01.pdf

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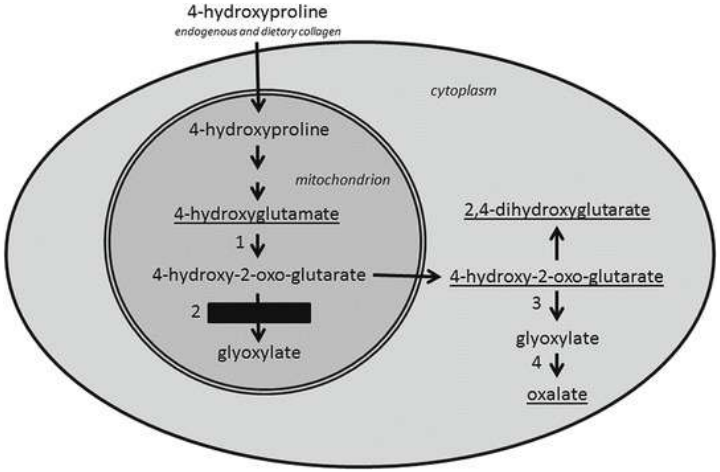
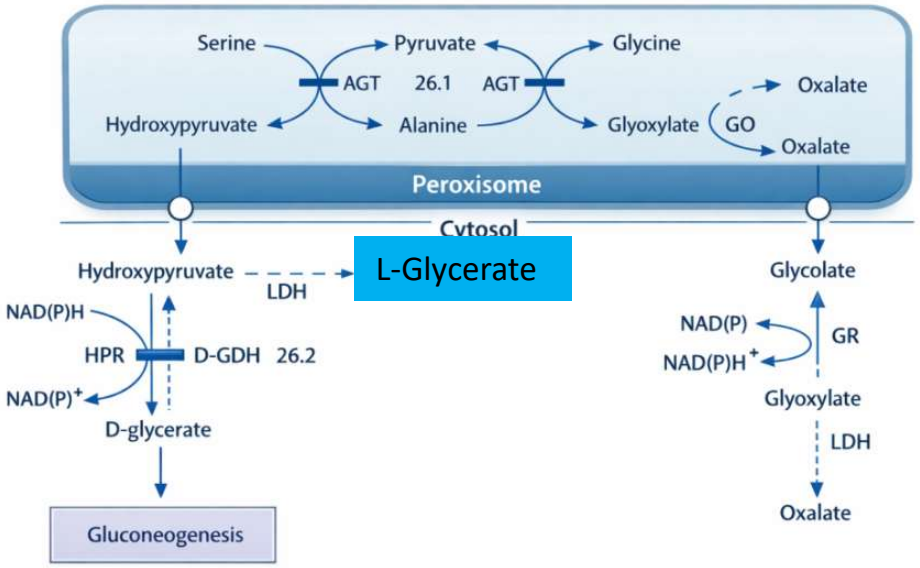
Analyte	Units	Added Amounts			
		Sample Pair 2025. 01 - 08	Sample Pair 2025. 02 - 07	Sample Pair 2025. 03 – 06	Sample Pair 2025. 04 – 05
4-OH-Glutamic acid	µmol/L	12.0	25.2	0.0	49.8
5-Aminolevulinic acid	µmol/L	5.5	14.9	0.0	29.6
5-OH indolacetic acid	µmol/L	28.1	58.3	97.8	12.9
Carnitine free	µmol/L	335	1.6	136	486
Creatine (9.1%)	**µmol/L**	**0.0**	**2000**	**1500**	**500**
Creatinine (4.9%)	**mmol/L**	**5.0**	**1.5**	**0.0**	**3.0**
D,L-Glyceric acid	µmol/L	280	928	140	0
Galactitol (9.3%)	**µmol/L**	**195**	**46.1**	**121**	**296**
Glycolic acid	µmol/L	124	0	49.6	201
Guanidinoacetate (8.2%)	**µmol/L**	**1.2**	**966**	**466**	**166**
Homocitrulline	µmol/L	0	2.0	10.1	5.1
Homogentisic acid	µmol/L	99	500	0	1000
Homovanillic acid	µmol/L	97	26.9	12.1	56.9
Lactic acid	mmol/L	2.9	5.7	8.9	0
L-Cystine	µmol/L	199	300	100	0
MPS (Chondroitin sulfate)	mg/L	97	17.0	47.1	147
Orotic acid	µmol/L	2.4	99	58	29
Oxalic acid	µmol/L	478	78	0	277
Pipecolic acid	µmol/L	19.5	29.8	40	2.2
Sialic acid	µmol/L	200	0	125	100
Succinylacetone	µmol/L	50	0.6	5.0	149
Sulfocysteine	µmol/L	32.9	10.1	3.0	19.8

Interlab CV < 10%

Analyte	CV% Interlab	Added Amounts			
		Sample Pair 2025. 01 - 08	Sample Pair 2025. 02 - 07	Sample Pair 2025. 03 – 06	Sample Pair 2025. 04 – 05
4-OH-Glutamic acid	41	12.0	25.2	0.0	49.8
5-Aminolevulinic acid	29	5.5	14.9	0.0	29.6
D,L-Glyceric acid	28	280	928	140	0
Homogentisic acid	32	99	500	0	1000
Oxalic acid	54	478	78.2	0	277
Succinylacetone	42	50	0.6	5.0	149

Hiperoxalurias (oxalate, 4-hydroxyglutamate and D.L-glycerate

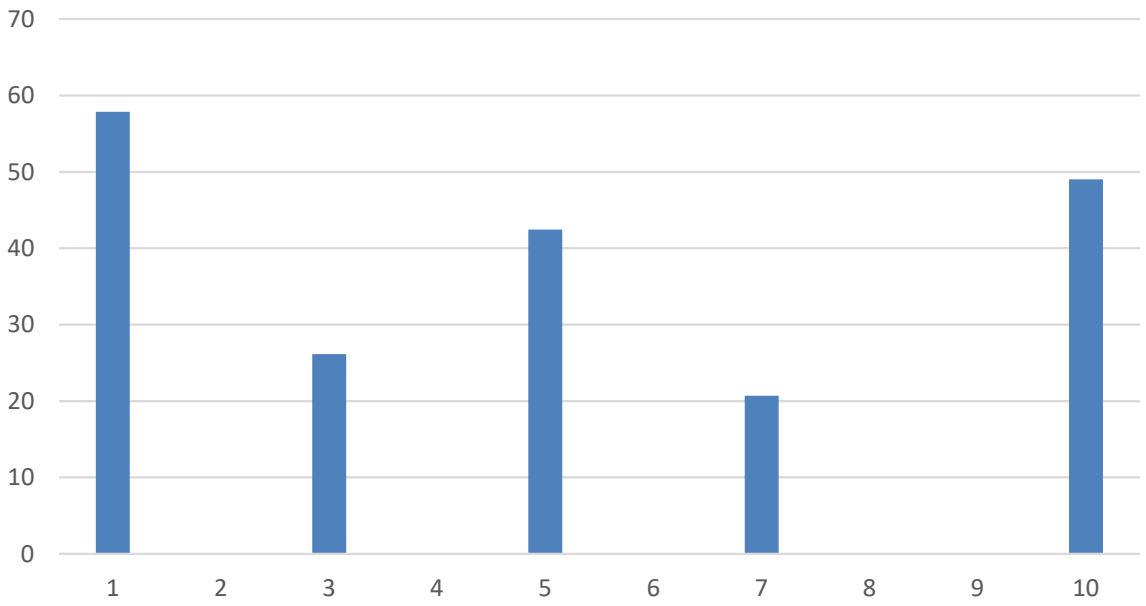




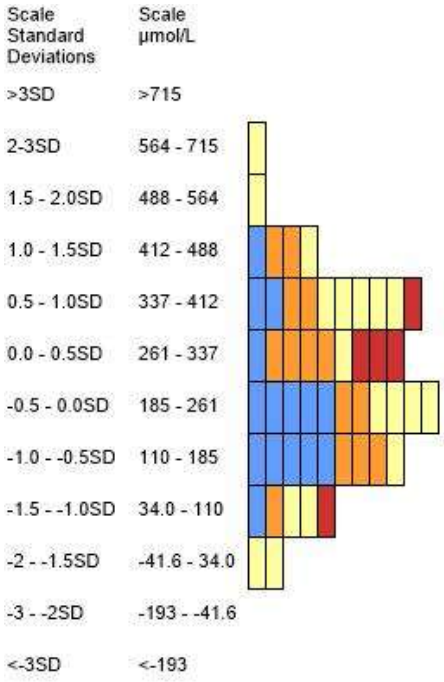
Pitt et al. JIMD Rep 2014:15:1-6.
von Schnakenburg, Latta. 2022.
In: Nenad Blau, Carlo Dionisi Vici, Carlos R. Ferreira eds.

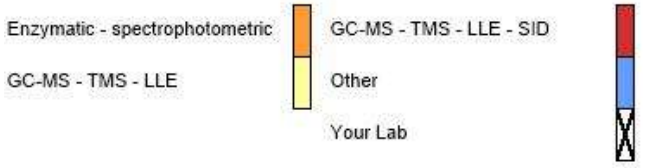
1) AGT alanine: glyoxylate aminotransferase (type I). ***Oxalic ↑↑ and glycolic (n/↑).***
2) GRHPR-related Glyoxylate reductase/hydroxypyruvate reductase deficiency (type II). ***Oxalic ↑↑ and L-glyceric (↑).***
3) 3) HOGA1-related Mitochondrial 4-hydroxy-2-oxoglutarate aldolase 1 deficiency deficiency (Type III). ***Oxalic, 4-OHglutamate and 4-Hydroxy-2-oxoglutaric acid (↑).***
4) SLC26A1-related Oxalate transporter deficiency ***(Oxalic acid ↑).***
5) GLYCTK-related D-glycerate kinase deficiency ***(D-glyceric ↑)***.

Oxalate



1	3	5	7	10
Mean = 261	Mean = 65	Mean = 33	Mean = 203	Mean = 253
SD = 151	SD = 17	SD = 14	SD = 42	SD = 124
%CV = 58	%CV = 26	%CV = 42	%CV = 21	%CV = 49
Target = 478	**Target = 78**	**Target = 0**	**Target = 278**	**Target = 478**



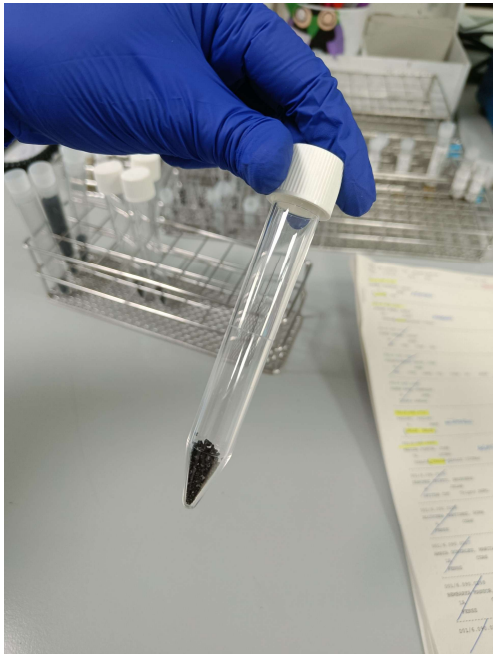


Oxalate (N=52). Spectrophotometric (14), GC/MS (18), 0ther (15).

Oxalate

Question: By which technique is majority of laboratories extracting oxalic acid from urine? Liquid-liquid extraction or maybe SPE?-

Probably Liquid-Liquid, since GC/MS is still the most common approach. However, consider the next the fully automated spectrometric analysis.

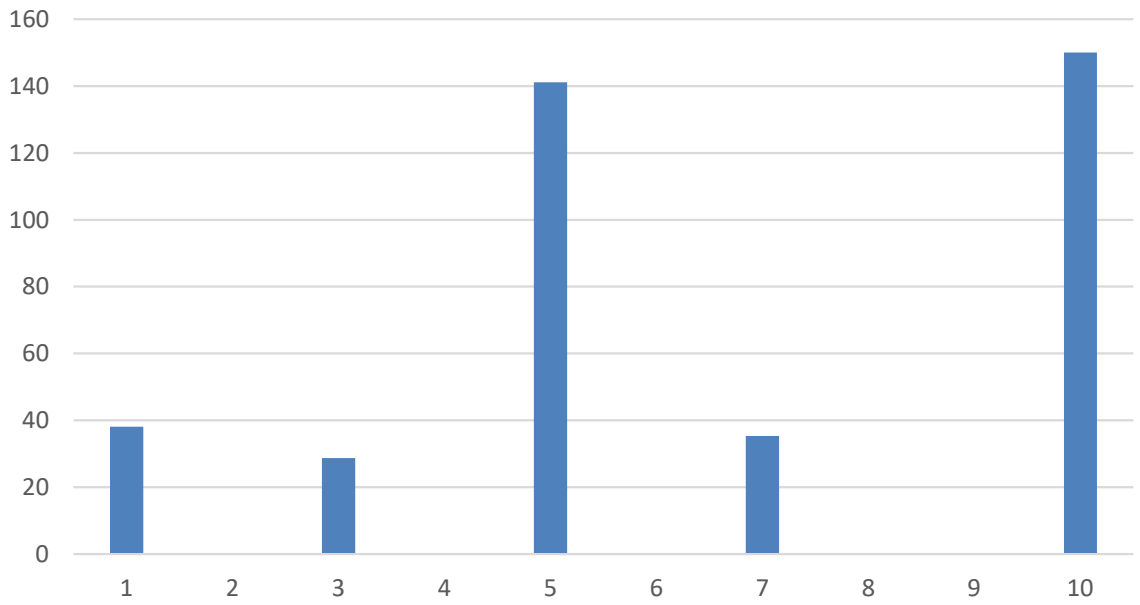


200 µL urine plus 200 µL buffer pH = 7.

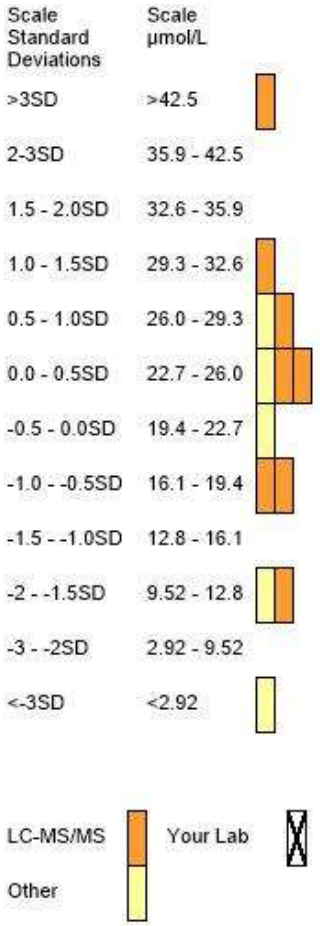
N=46
Pairs 1 – 8 (Target 478). 6 Labs > 400.
Pairs 2 – 7 (Target 78). 27 Labs > 65
Pairs 4 – 5 (Target 278). 17 Labs > 225.

Oxalate soluble in acidic medium

4-hydroxyglutamic acid (µmol/L)

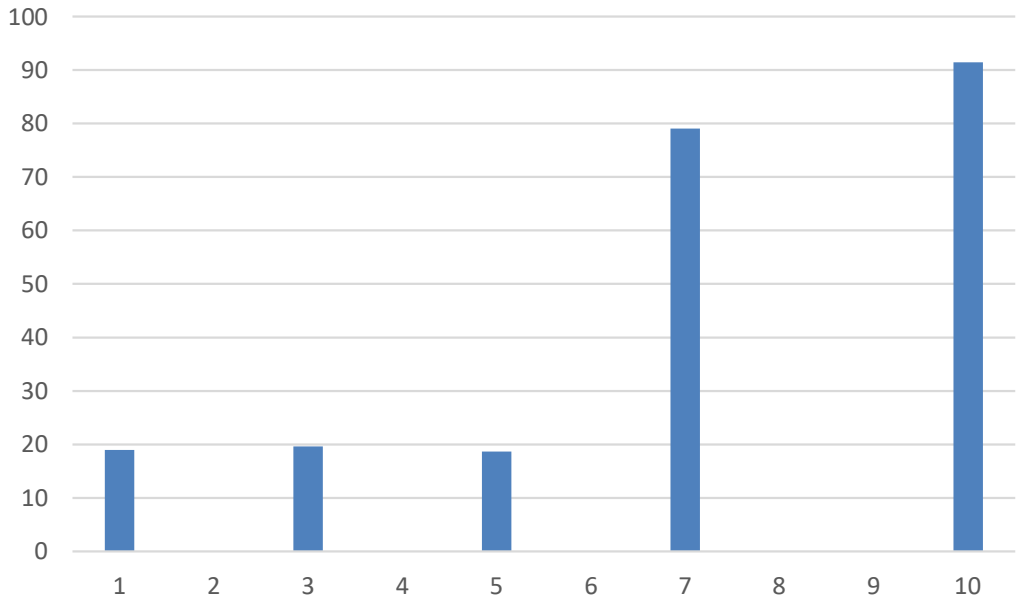


Mean = 10.5	Mean = 23	Mean =0.17	Mean = 51	Mean = 0.2
SD = 4	SD = 6.6	SD = 0.24	SD = 18	SD = 0.3
%CV = 38	%CV = 29	%CV = 141	%CV = 35	%CV = 150
Target = 12	**Target = 25**	**Target = 0**	**Target = 50**	**Target = 0**

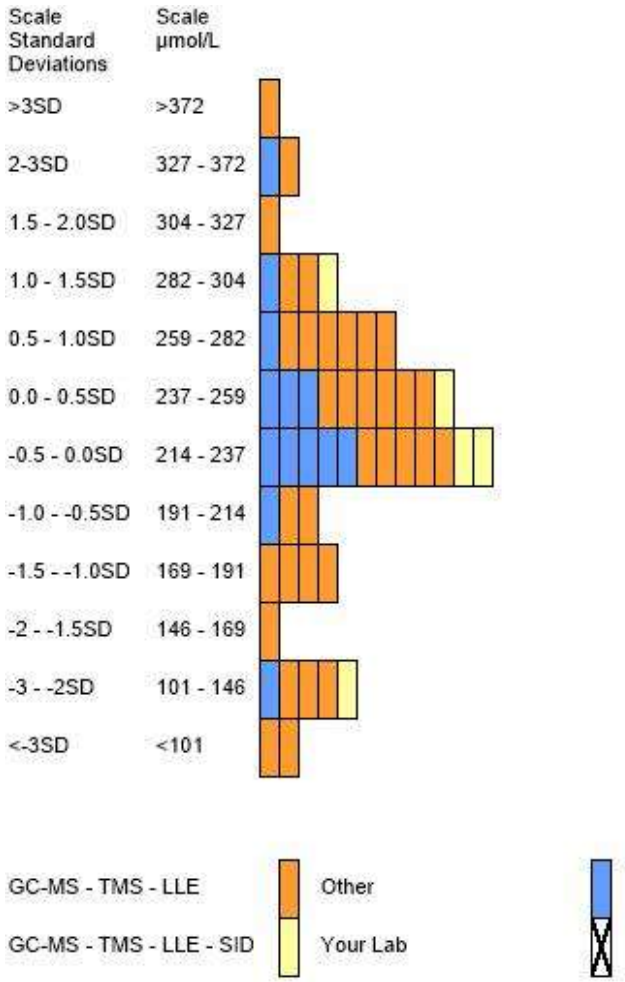


(n=14): 8 LC-MS, 6 others

D,L-glyceric acid



Mean = 237	Mean = 769	Mean =118	Mean = 4,3	Mean = 3,5
SD = 45	SD = 151	SD = 22	SD = 3,4	SD = 3,2
%CV = 19	%CV = 20	%CV = 19	%CV = 79	%CV = 91
Target = 280	**Target = 930**	**Target = 140**	**Target = 0**	**Target = 0**



(n=48): GC/MS (35) Others (13)

5-aminolevulinic acid (5-ALA: µmol/L)

Porphyrin:

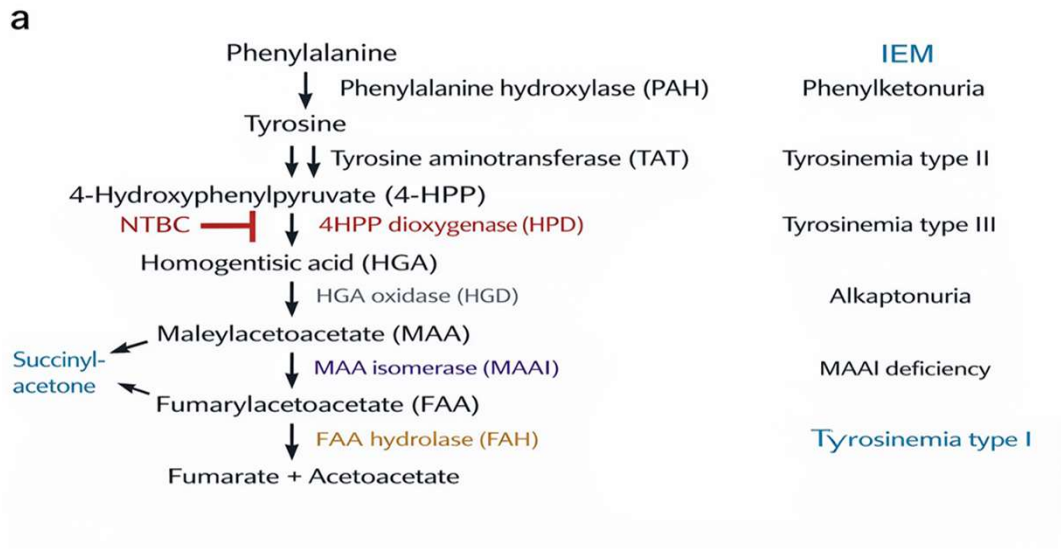
- Inherited acute porphyria (ALAD deficiency)
- Secondary inhibition of ALAD due to fumarylacetoacetase deficiency,

lead intoxication, alcoholism and pregnancy.

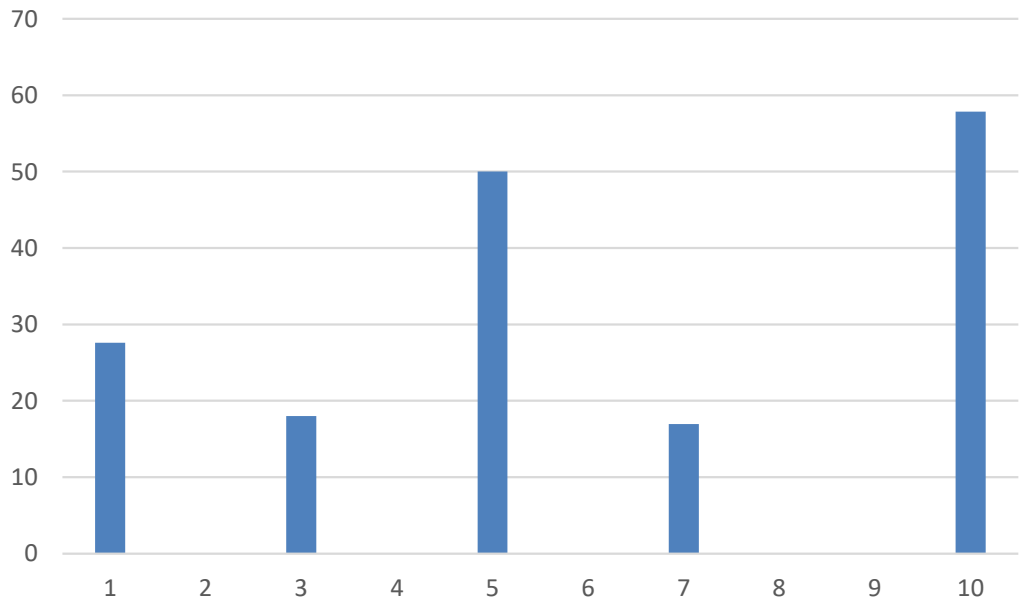
5-ALA

↓ *ALA dehydratase*

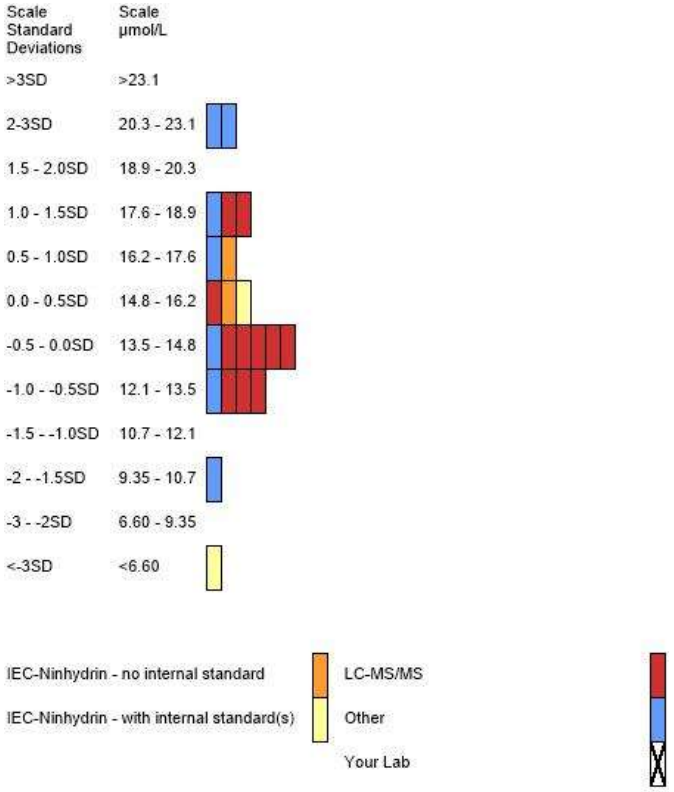
Porphobilinogen



5-aminolevulinic acid (µmol/L)

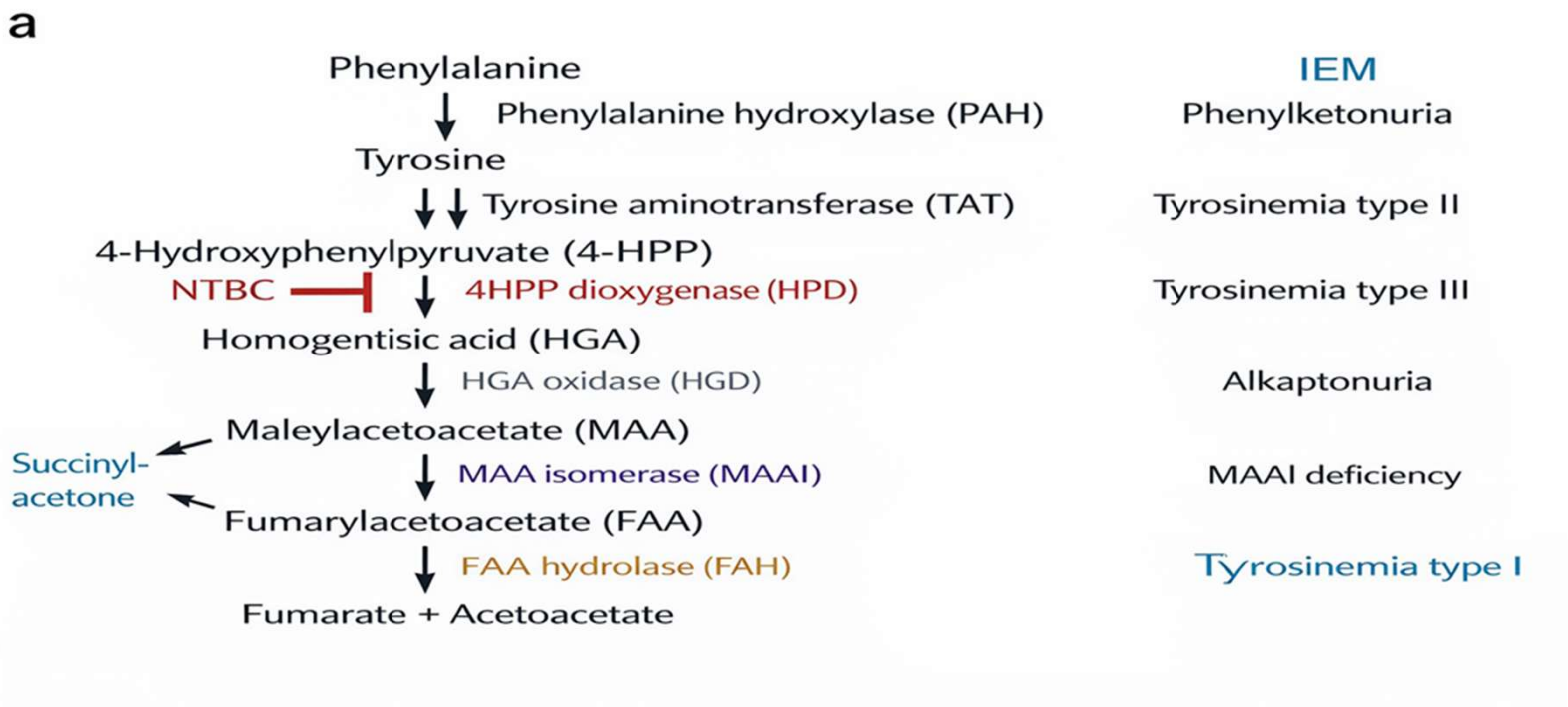


Mean = 5.8	Mean = 15	Mean =1.4	Mean = 23	Mean = 1.4
SD = 1.6	SD = 2.7	SD = 0.7	SD = 3.9	SD = 0.81
%CV = 28	%CV = 18	%CV = 50	%CV = 17	%CV = 58
Target = 5.5	**Target = 14.9**	**Target = 0**	**Target = 30**	**Target = 0**

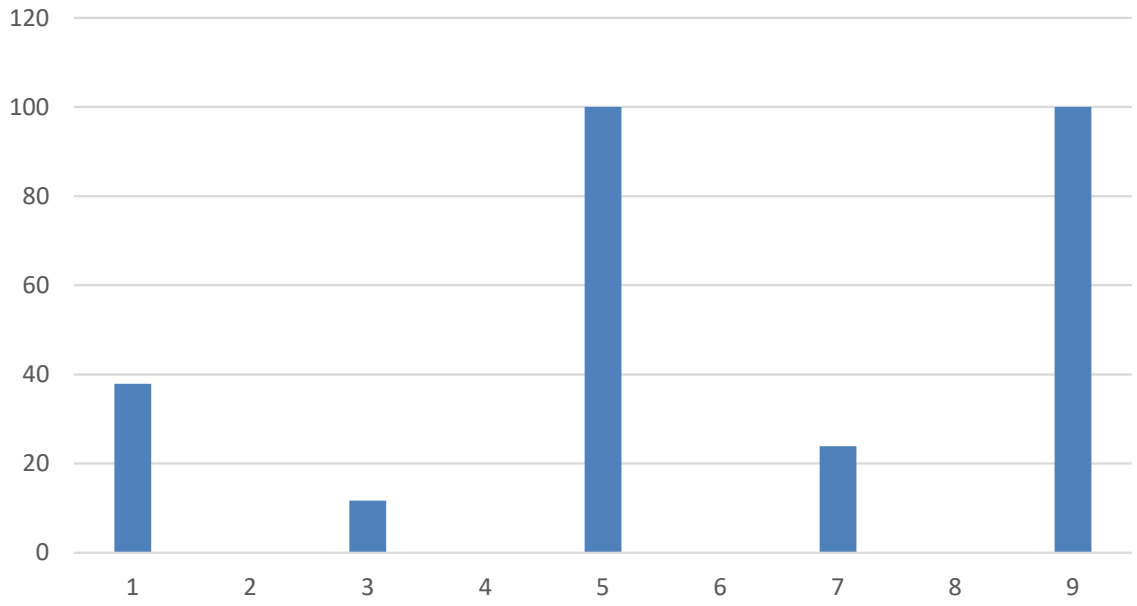


(n=23): IEC (4). LC-MS (11). Others (7).

Homogentisic acid

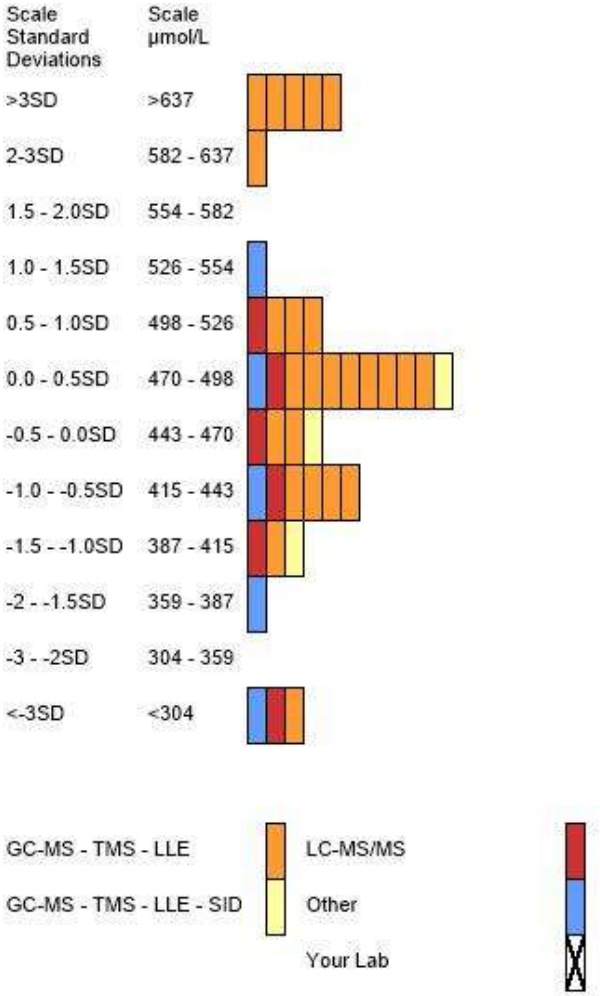


Homogentisic acid



Mean = 66	Mean = 470	Mean =0,008	Mean = 890	Mean = 0,003
SD = 25	SD = 55	SD = 0.1	SD = 213	SD = 0.3
%CV = 38	%CV = 12	%CV > 100	%CV = 24	%CV > 100
Target = 99	**Target = 500**	**Target = 0**	**Target = 1000**	**Target = 0**

(n=39). GC/MS (28). LC/MS (6). Others (5).

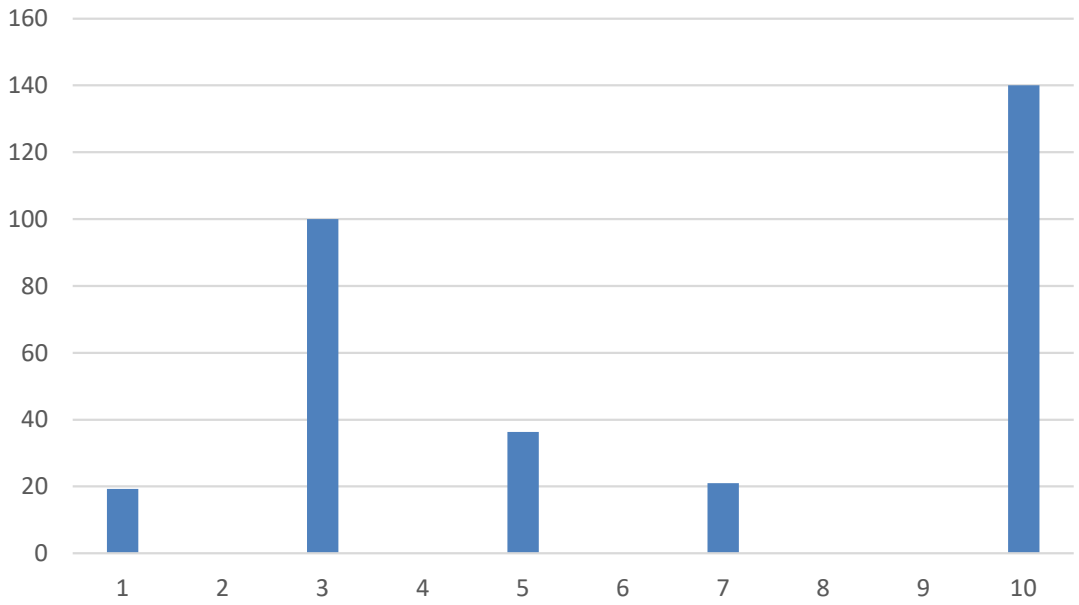


Homogentisic acid

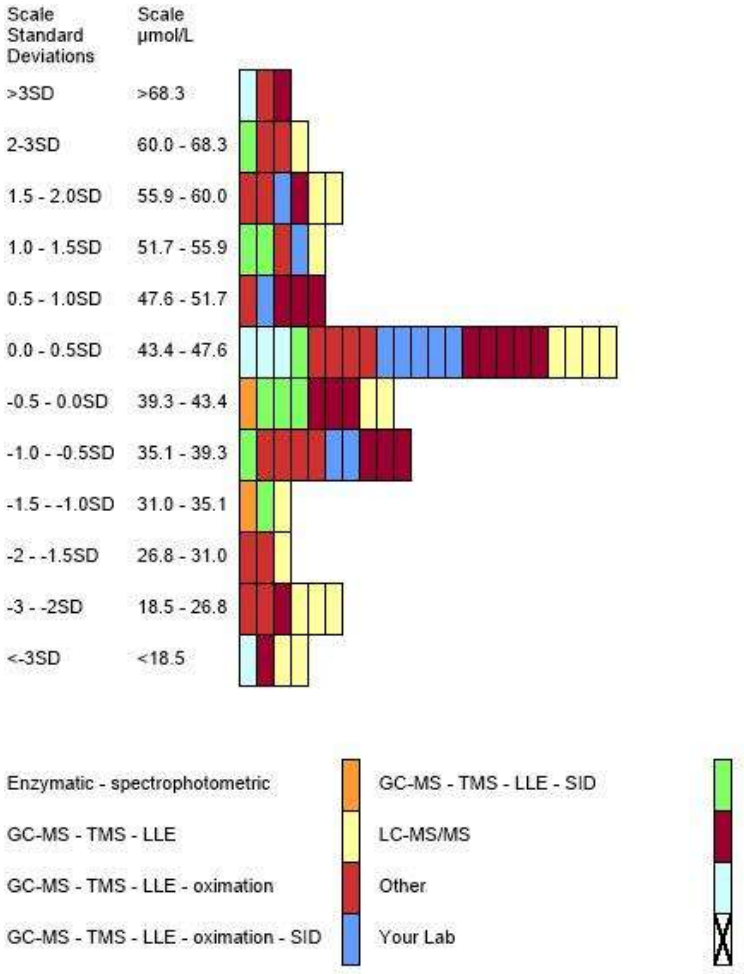
Question: The Stability of analytes. HGA is not stable long term in DI water matrix so our lab is testing whether making the SAU EQA in acidified DI water will make any difference, hence we ordered two boxes of these EQA to test?!

Pairs 8-1: 66 → 64 (Target 100)
Pairs 7-2: 470 → 450 (Target 500)
Pairs 6-3: 0
Pairs 5-4: 890 → 914 (Target 1000)

Succinylacetone



Mean = 43	Mean = 0.5	Mean = 4,4	Mean = 124	Mean = 0.5
SD = 8.3	SD = 0.5	SD = 1.6	SD = 26	SD = 0.7
%CV = 19	%CV = 100	%CV = 36	%CV = 21	%CV = 49
Target = 50	**Target = 0.6**	**Target = 5**	**Target = 149**	**Target = 0.6**

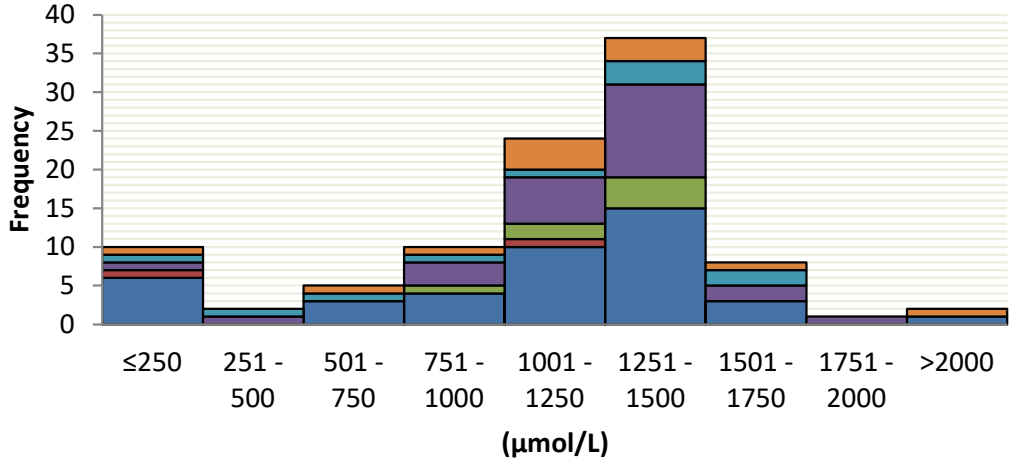


(n=80). No clustering.

Low recovery for the highest value

L-Cystine (clinical sample)

1,210 umol/L



Bin	IEC - Ninhydrin	LC-MS	LC-MS - derivatised	LC-MS/MS	LC-MS/MS - derivatised	Other (please specify)
≤250	6	1	0	1	1	1
251 - 500	0	0	0	1	1	0
501 - 750	3	0	0	0	1	1
751 - 1000	4	0	1	3	1	1
1001 - 1250	10	1	2	6	1	4
1251 - 1500	15	0	4	12	3	3
1501 - 1750	3	0	0	2	2	1
1751 - 2000	0	0	0	1	0	0
>2000	1	0	0	0	0	1

L-Cystine (SAU scheme)

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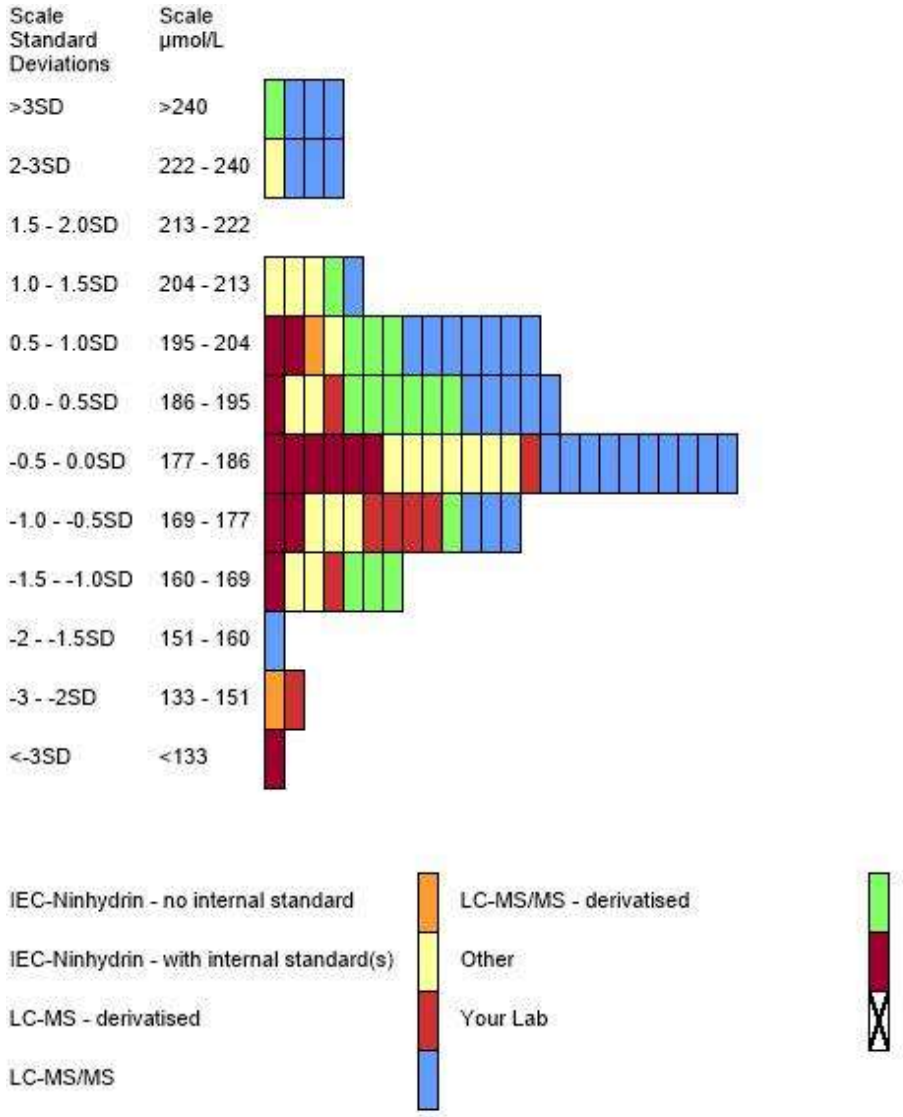
(n=90)

Interlab CV → 11.7 %
Recovery → 59%.
Target 100 µmol/L (90).
Target 200 µmol/L (140).

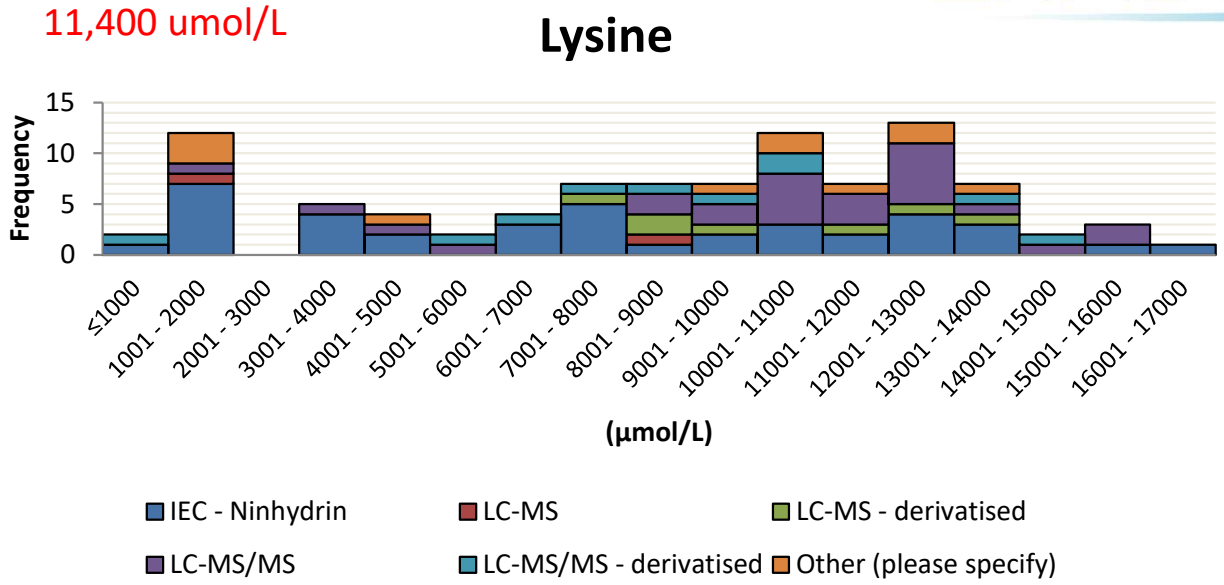
Target 300 µmol/L (186)
Only 4 labs > 240



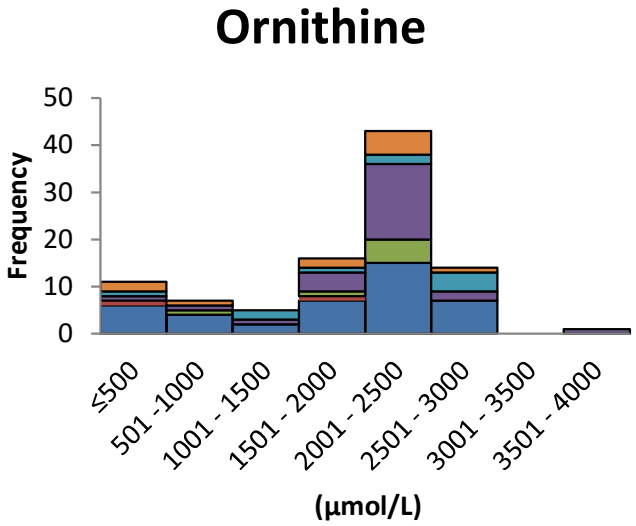
Urine amino acids (clinical sample)

Main complain
Lack of volume

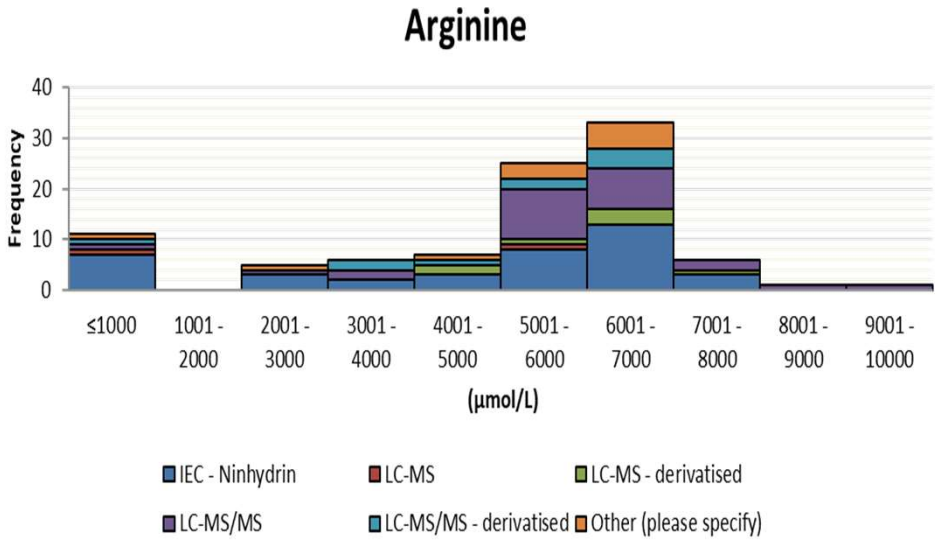
11,400 umol/L



2,220 umol/L

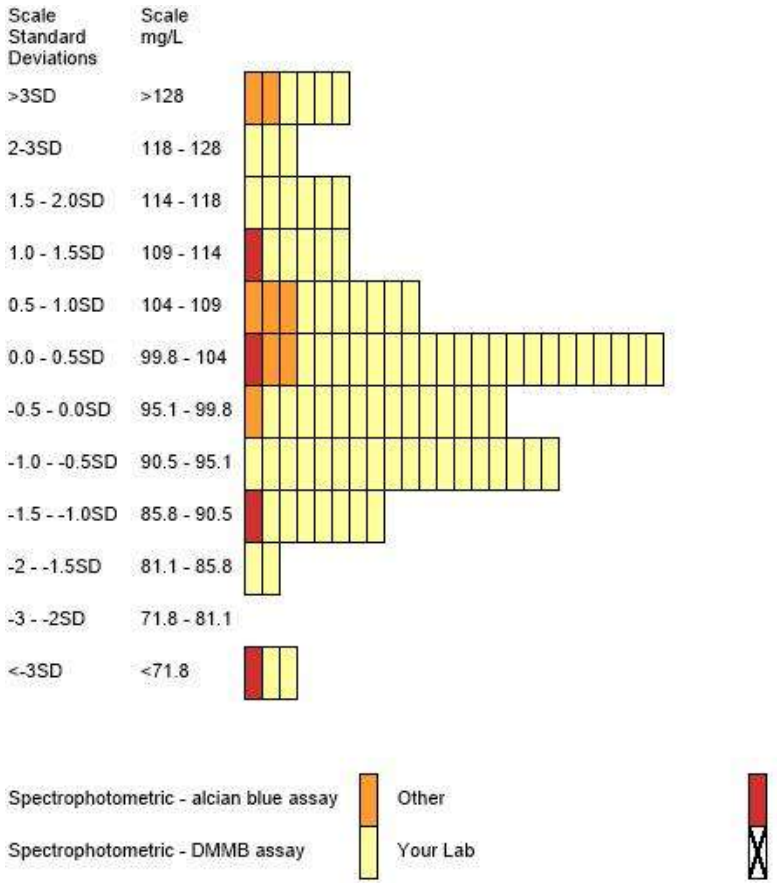


5,750 umol/L



Other Questions

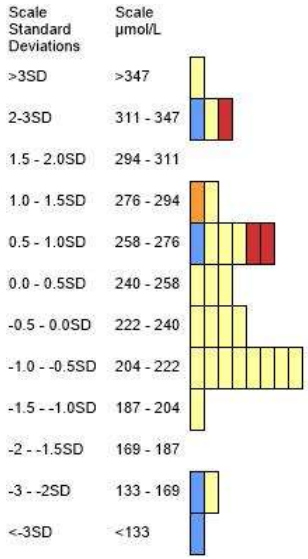
Question: How does the alcian blue colorimetric method compare to the dimethylene blue colorimetric method?

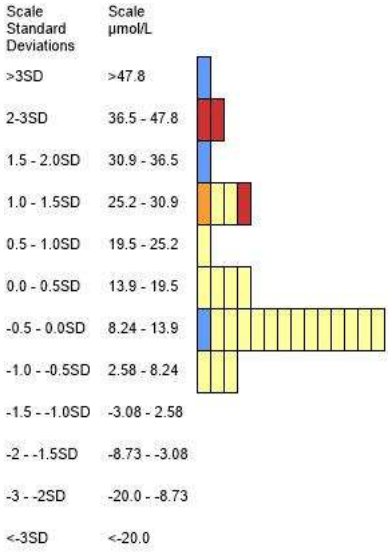


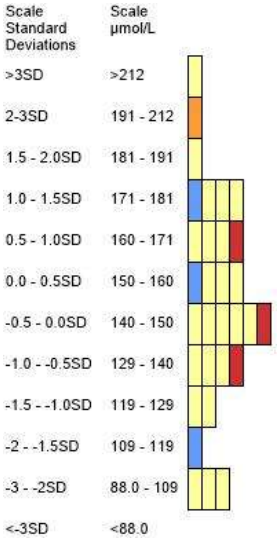
Sialic acid

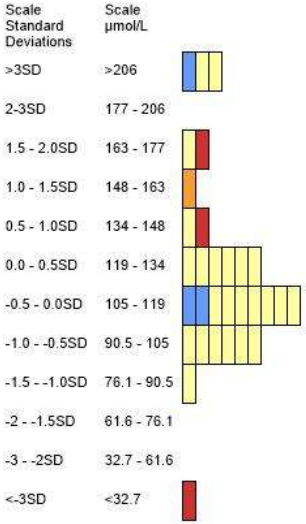
Technical of urine sialic acid quantitation. It seems there are various methods used among participant, what are the pros and limitation of each method. We noticed that our method is always constant bias compared to group mean.

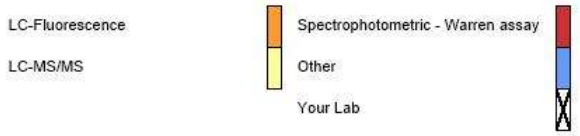
Interlab CV = 18%











General questions

- How do you establish the calibration curves for biomarkers given the control concentrations can range from extremely low to very high? Glc4 will also be included in urine quality tests? And lyso-GM2?

SAS and SAU samples shall be processed following the same procedure as patient samples. An adequate linearity interval must be ensured; if results fall outside this interval, the necessary corrections shall be applied, including dilution when required due to high concentration.

Glc4 → Pompe in educational Kit for oligossaccharidoses (MCA). Very expensive.

Lyso-GM → is already in LIS.

- Has there been a change in the SAU EQA sample matrix prior to the 2024 scheme? I have noticed interference in the cystine assay which now requires a dilution to run.

No, no changes (MCA answer)

General questions

- Since the scheme involves a wide range of metabolites, is there a recommended process flow to enable efficient workflow/diagnosis?

No, but it is a good idea.

For SAS probably it is not necessary, at least after the assessment of the performance of the metabolites moved to LIS that are the most unstable…

For SAU, yes. Specially to treat the sample to prevent L-cystine and oxalate precipitation. The spiked samples probably has less solubility that the natural ones.

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THANKS

Remember the annual survey for adding new ideas.