ERNDiM

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Quality Assurance in Laboratory Testing for IEM

Feedback from 24 Schemes

- 6 samples per participant sent across 3 centres, each centre typically has a unique set of samples except for one common sample in 2024 (2 sites) and one in 2025 (3 sites)

  QLOU Sheffield

  QLOU Barcelona

  QLOU Heidelberg

Scientific advisors

- QLOU Sheffield
  - Camilla Scott

- QOU Barcelona
  - Dr. Judit García Villoria

- QLOU Heidelberg
  - Dr. Joachim Janda

QLOU schemes

- Samples are analysed and reported in two cohorts, A, B, C in the Spring. C,D,E in the late Summer.
- Results are uploaded onto the CSCQ website and scored by the SA's for each scheme.
- Results are second scored by another SA from one of the other two centres.
- Results, educational sample and critical errors are discussed with the wider group at the SAB in the Autumn of each year.

ERNDIM QLOU participants in 2024

Nº Registrations/year	QLOU Barcelona (EB)	QLOU Heidelberg (HD)	QLOU Sheffield (US)	TOTAL
2024	**77**	**77**	**75**	**229**
2023	77	74	73	224
2022	74	76	74	224
2021	73	74	74	221

Scoring

A	Analytical Performance	Correct interpretation of the of the profile as normal/abnormal and Correctly assigning the abnormal profile type (if relevant)	2
		Correct interpretation of the profile as normal/abnormal, or Correctly assigning the abnormal profile type (if relevant)	1
		Unsatisfactory or misleading (in some instances will be evaluated also as a critical error – See DOC2305), or No result submitted	0
I	Interpretative proficiency	Good (diagnosis was established and appropriate further tests were recommended)	2
		Helpful but incomplete	1
		Misleading/wrong diagnosis (will be most likely evaluated also as a critical error – see DOC2305), or No result submitted	0

The scoring can vary in different samples of the same disease because it depends on the difficulty of the diagnosis. It is very important to complete the further studies, as they can be helpful in complicated cases.

Critical Errors

- Discussed in SAB meeting
- Main criteria:

➢Failure to recognize a predefined set of diagnoses.

➢Missing a diagnosis when proficiency for that EQA sample is >95%.

➢Drawing misleading conclusions (e.g., missing a diagnosis or, even worse, making an incorrect diagnosis).

➢Identifying a normal sample as having an IEM, in specific cases, depends on the impact.

Fill correctly the key metabolites or normal sample

Put normal profile in the box and in qualitative results normal

Put the altered/key metabolite name in the box and select elevated, low, or normal. QUANTIFICATION IS NO MANDATORY

Results entry

Survey **21-05-OUS** - Laboratory

Selected sample: **QLOU-US-2021-A**

Select another sample →

Clinical picture	**Speech & language delay**
	Sex: F

Samples received on (yyyy-mm-dd):

Step 1 : Selection of used analytes/me

Selection

Step 2 : Analytical results input

1. Pre-investigations (0/0)
2. Organic acids analysis (0/0)

Step 3 : Interpretation input

Interpretation (to be entered)

Step 4 : Further lab investigations

Recommendations (to be entered)

Step 5: File upload

File upload

Step 6: Proof reading

Proof reading

changing page (other survey or sample)

Back to **Result entry**

Analyte	Method	Key Metabolite	Quant. result	Unit	Evaluation
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Normal profile	******	mmol/mol creat	Normal
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	3-hydroxypropionate	100	mmol/mol creat	Elevated
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	methylcitric acid	2	mmol/mol creat	Normal
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered
Organic acids column chromatography	Method 1/GC-MS ; Ethylacetate ; No ; Silylation	Please specify key metabolite	******	mmol/mol creat	To be entered

This comments are not evaluated

Comments:

Proficiency across all the samples Sheffield Scheme 2024:

- **A. Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) 94%**
- B. Maple Syrup Urine Disease (MSUD) *Incorrect clinical info on website 96 %
- C. Normal *Incorrect clinical info on website*95%
- D. Ethylmalonic Aciduria (EMA)97%
- E. Methylmalonic Aciduria (MMA)100%
- **F. Aromatic L-amino decarboxylase (AADC) deficiency 84% (Heidelberg: 64%)**

Sample A - MCADD

Clinical features:

- Presumptive positive of Newborn Screening, well patient.

Analytical findings:

- Increased hexanoyl, and suberyl glycine.

Correct Diagnosis:

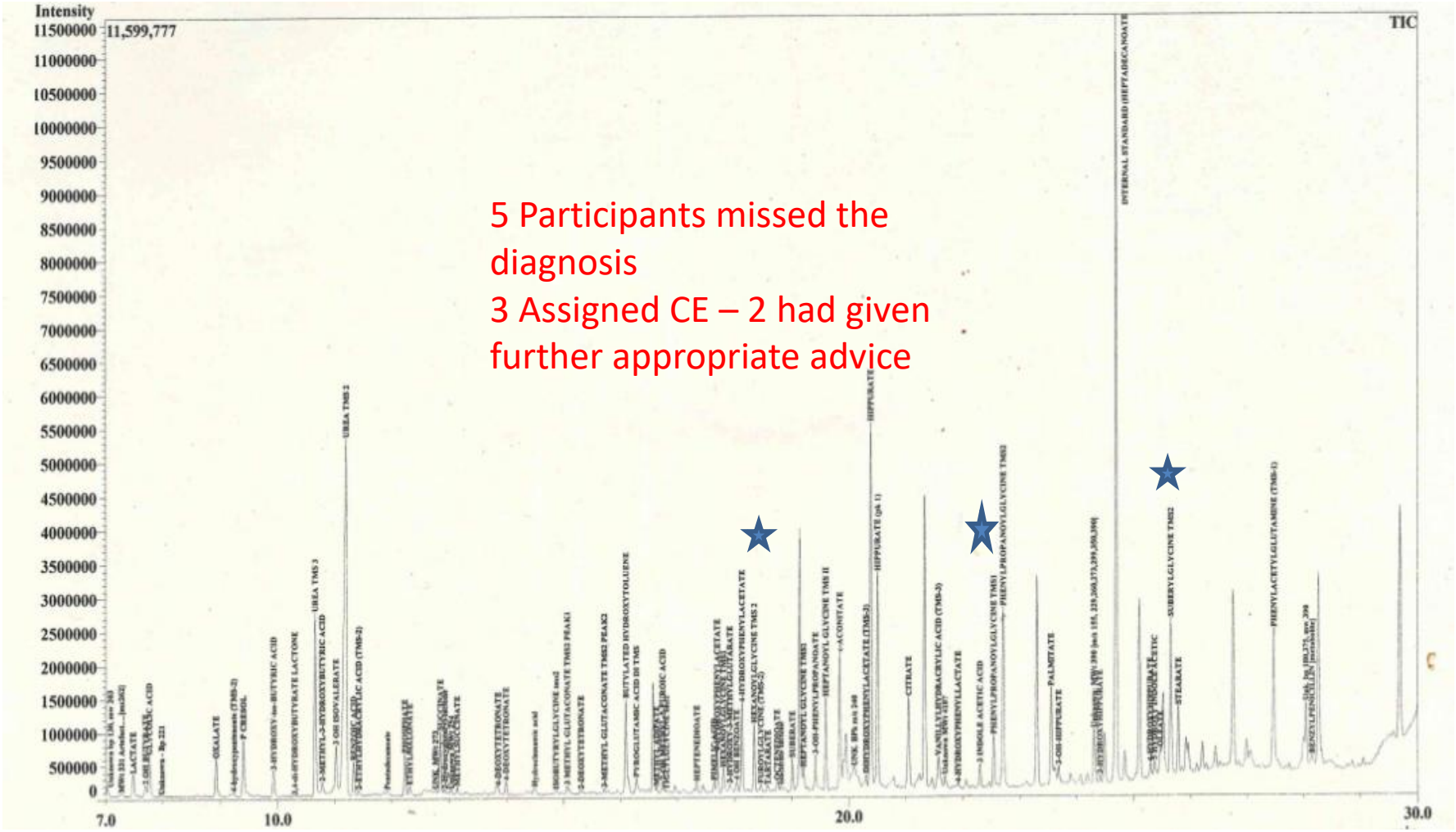
- MCADD

Acceptable recommendations:

- Molecular analysis, urine organic acids, metabolic referral.

% of participants with correct diagnosis:

- 94%


Intensity
11,599,777
TIC
5 Participants missed the diagnosis
3 Assigned CE – 2 had given further appropriate advice
7.0
10.0
20.0
30.0

Sample B – Maple Syrup Urine Disease

Clinical features:

- Should have been: *Developmental delay 4-year-old-male*
- The wrong clinical details were sent out with this sample: *Recurrent abdominal pain, 8-year-old female on a diary free diet.*

Analytical findings:

Branched-chain oxo and hydroxyacids.

(It is noted that this was a dilute sample however the key metabolites were still present in the profile.)

Correct Diagnosis:

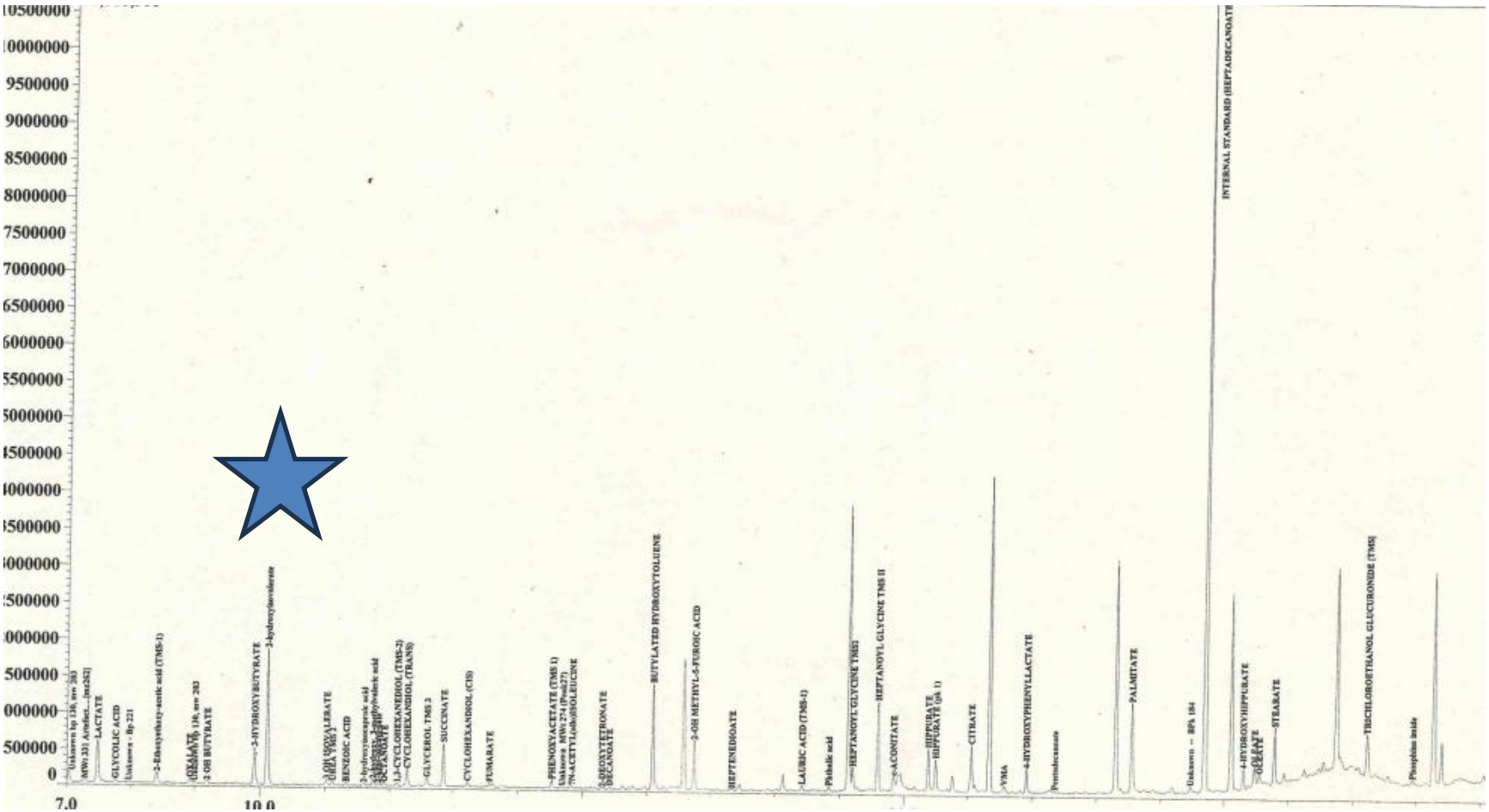
- MSUD

Acceptable recommendations:

- plasma amino acids and molecular analysis of E1 alpha BCKDH1A, E1 beta BCKDH1B, E2 DBT, E3 DLD.

% of participants with correct diagnosis:

90 percent of participants suggested Maple Syrup Urine Disease of variable phenotype (intermittent or intermediate form) was the most likely or an alternative diagnosis. Full interpretative marks were given to all participants who suggested MSUD as a first or alternative diagnosis 96 percent.


LACTATE
GLYCOLIC ACID
2-OH BUTYRATE
3-HYDROXYBUTYRATE
3-hydroxyisovalerate
BENZOIC ACID
CYCLOHEXANEDIOL (TRANS)
GLYCEROL TMS 3
SUCCINATE
CYCLOHEXANEDIOL (CIS)
FUMARATE
PHENOXYACETATE (TMS 1)
DECANOATE
BUTYLATED HYDROXYTOLUENE
2-OH METHYL-6-FUROIC ACID
HEPTENEDIOATE
LAURIC ACID (TMS-1)
HEPTANOYL GLYCINE TMS II
ACONITATE
HIPPURATE
CITRATE
VMA
PALMITATE
INTERNAL STANDARD (HEPTADECANOATE
4-HYDROXYHIPPURATE
STEARATE
TRICHLOROETHANOL GLECURONIDE (TMS)
7.0
10.0

Sample C - Normal

Clinical features:

- Should have been: *Recurrent abdominal pain, 8-year-old female on a diary free diet.*
- The wrong clinical details were sent out with this sample: Extrapyramidal signs & Chronic Kidney Disease 2-year-old male

Analytical findings:

- No analytical findings (4 participants found a trace of MMA)

Correct Diagnosis:

- Normal (retrospective Vitamin B12 deficiency)

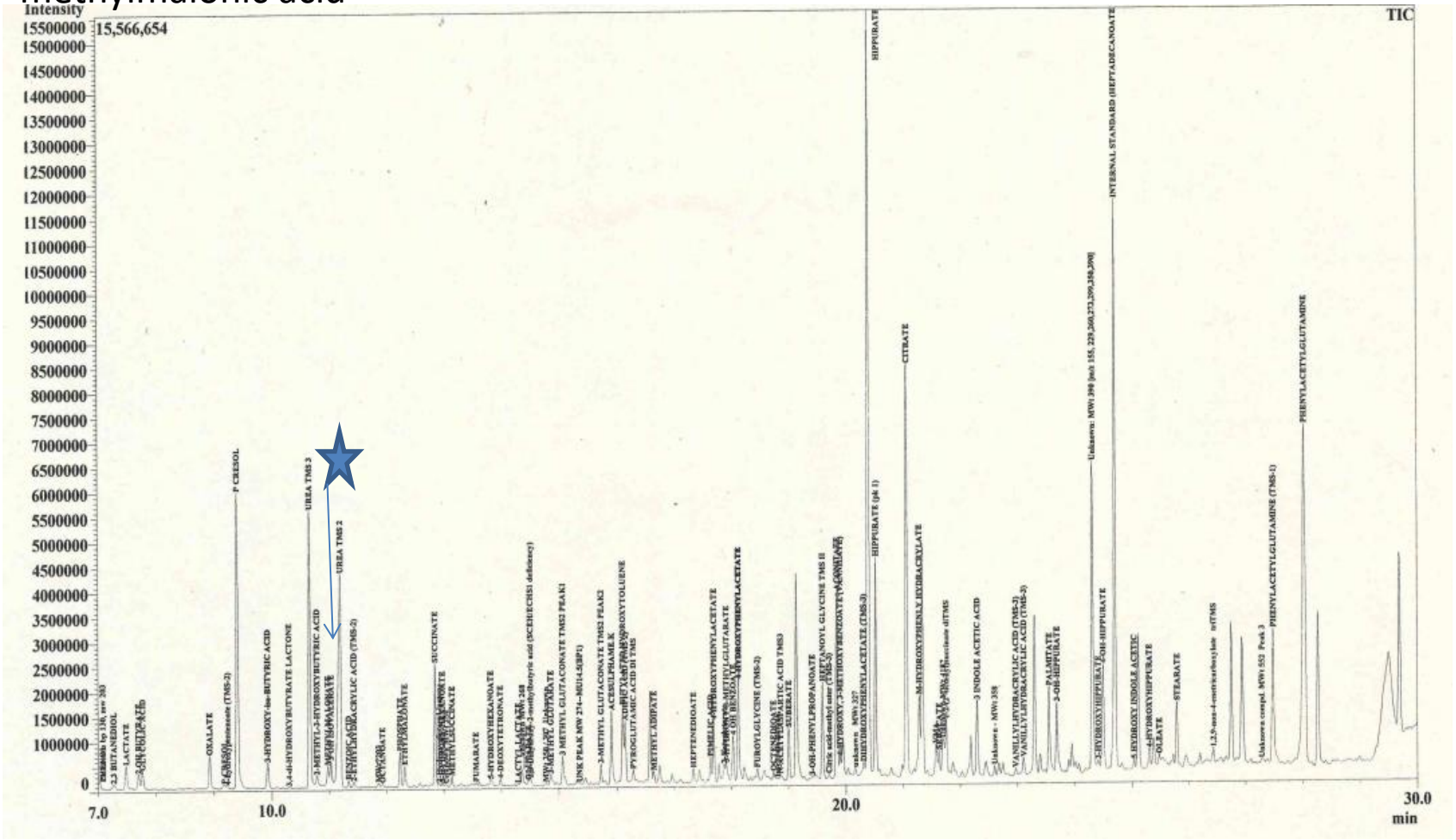
Acceptable recommendations:

- None required (B12 assessment)

% of participants with correct diagnosis:

- 95 percent (although marks were not removed for MMA or other mild/secondary disorders due to the issues with the clinical details and the retrospective diagnosis)

Four participants suggested vitamin B12 deficiency was a consideration. Because biochemically this child had low vitamin B12 levels marks were awarded for reports of trace amounts of methylmalonic acid



Sample D – Ethylmalonic aciduria

Clinical features:

- Learning difficulties 6-year-old male

Analytical findings:

- Increased ethylmalonic acid

Correct Diagnosis:

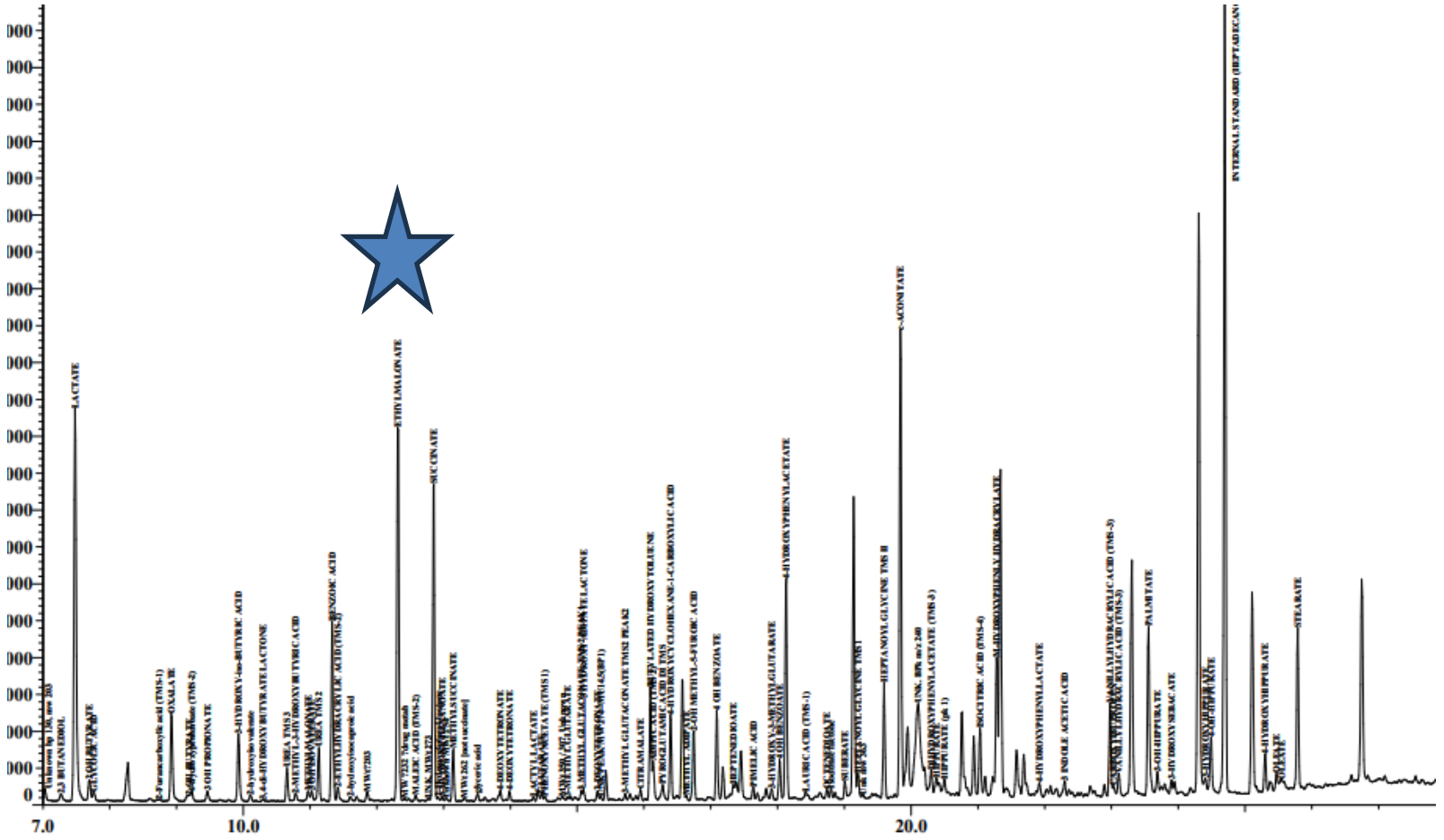
- Ethylmalonic aciduria unknown cause

Acceptable recommendations:

- Plasma acylcarnitines and molecular analysis of the various genes associated with increased excretion of ethylmalonic acid (ACADS, ETHE1, ETFDH/A/B etc).

% of participants with correct diagnosis:

- 97%


7.0
10.0
20.0

E. Methylmalonic Aciduria (MMA)

Clinical features:

- Extrapyramidal signs & Chronic Kidney Disease 2-year-old male.

Analytical findings:

- Increased MMA and increased methyl citrate

Correct Diagnosis:

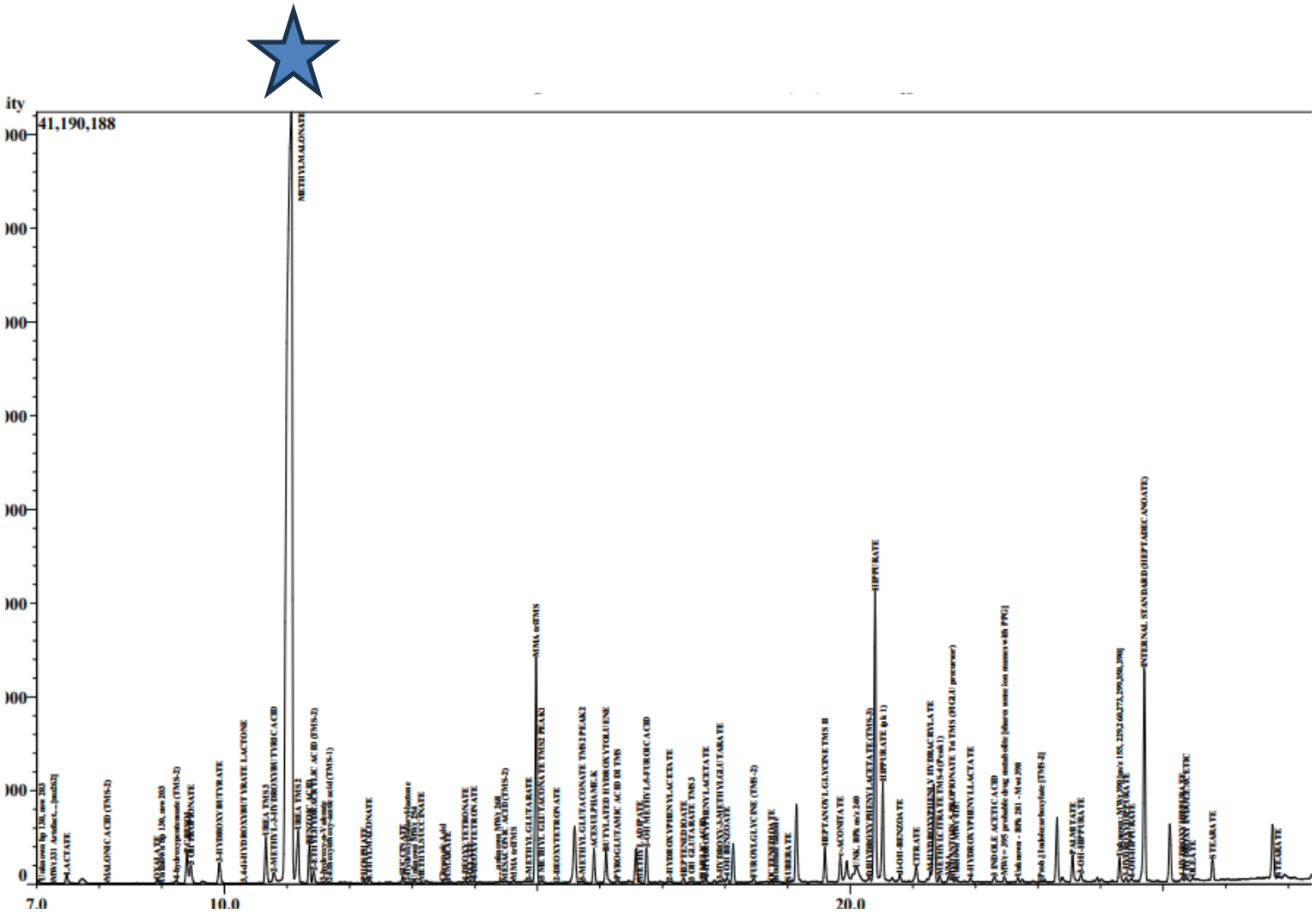
- MMA mutase

Acceptable recommendations:

- Urgent metabolic referral, molecular analysis

% of participants with correct diagnosis:

- 100 %


ity
41,190,188
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0
7.0
10.0
20.0
METHYLMALONATE
HIPPURATE

Sample F - AADC

Clinical features:

Predominantly truncal hypotonia and intermittent dystonic posturing. On treatment. 8-year-old male.

Analytical findings:

- GC-MS analysis of the sample reveals an elevated concentration of vanillactic acid (VLA) while vanillylmandelic acid (VMA) is in the lower normal concentration range. The diagnostic ratio VLA/VMA is grossly elevated

Correct Diagnosis:

- AADC on Treatment

Acceptable recommendations:

- Recommendations included biogenic amines (CSF), enzyme studies and molecular confirmation by mutational analysis of the DDC gene. Referral to the metabolic team

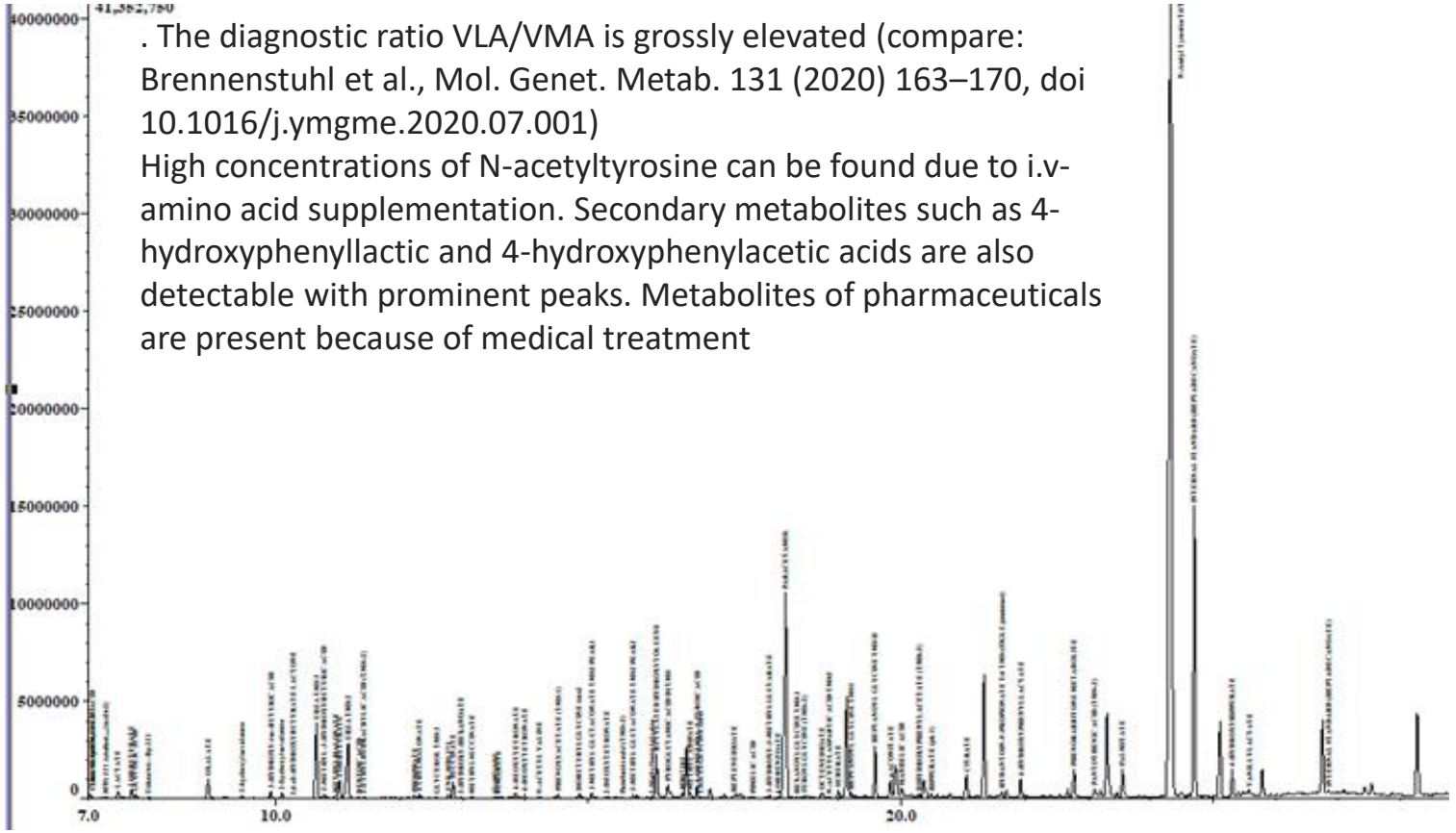
% of participants with correct diagnosis:

- 84% Sheffield 64% Heidleburg

Difficult sample 2024 – AADC common sample to Sheffield and Heidelberg participants.

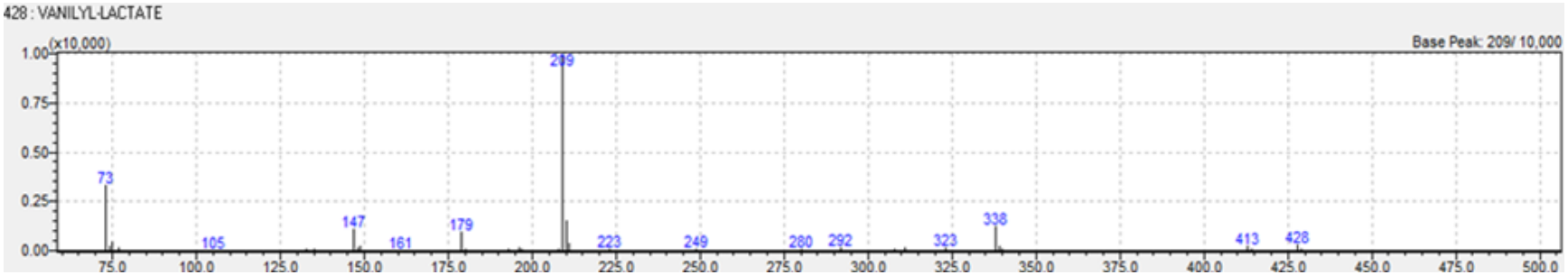
- This was a difficult sample taken from a patient with previously diagnosed Aromatic L-amino acid decarboxylase deficiency having undergone intracerebral gene therapy with the additional complication of parenteral nutrition.
- The overall proficiency for this difficult sample however was good at eighty-four percent (Sheffield) and sixty-four percent (Heidelberg),
- A significant improvement compared to the last circulation of an AADC sample in which diagnostic proficiency was only 56 percent Sheffield (2020) and 40% Heidelberg (2021)

AADC on treatment



. The diagnostic ratio VLA/VMA is grossly elevated (compare: Brennenstuhl et al., Mol. Genet. Metab. 131 (2020) 163–170, doi 10.1016/j.ymgme.2020.07.001)
High concentrations of N-acetyltyrosine can be found due to i.v-amino acid supplementation. Secondary metabolites such as 4-hydroxyphenyllactic and 4-hydroxyphenylacetic acids are also detectable with prominent peaks. Metabolites of pharmaceuticals are present because of medical treatment

Vanilyl lactate



Sheffield Scheme 2025:

- Tyrosinaemia Type 1 (Treated)
- 3-Methylcrotonyl-CoA carboxylase deficiency
- Pyroglutamic Aciduria (Acquired)
- Fumarase deficiency
- Succinic semialdhehyde DH deficiency (SSADH)
- Normal (small peak of glycerol)

Sample B

Clinical details provided to participants:

Diagnosed in childhood. Adult – 37-year-old now well.

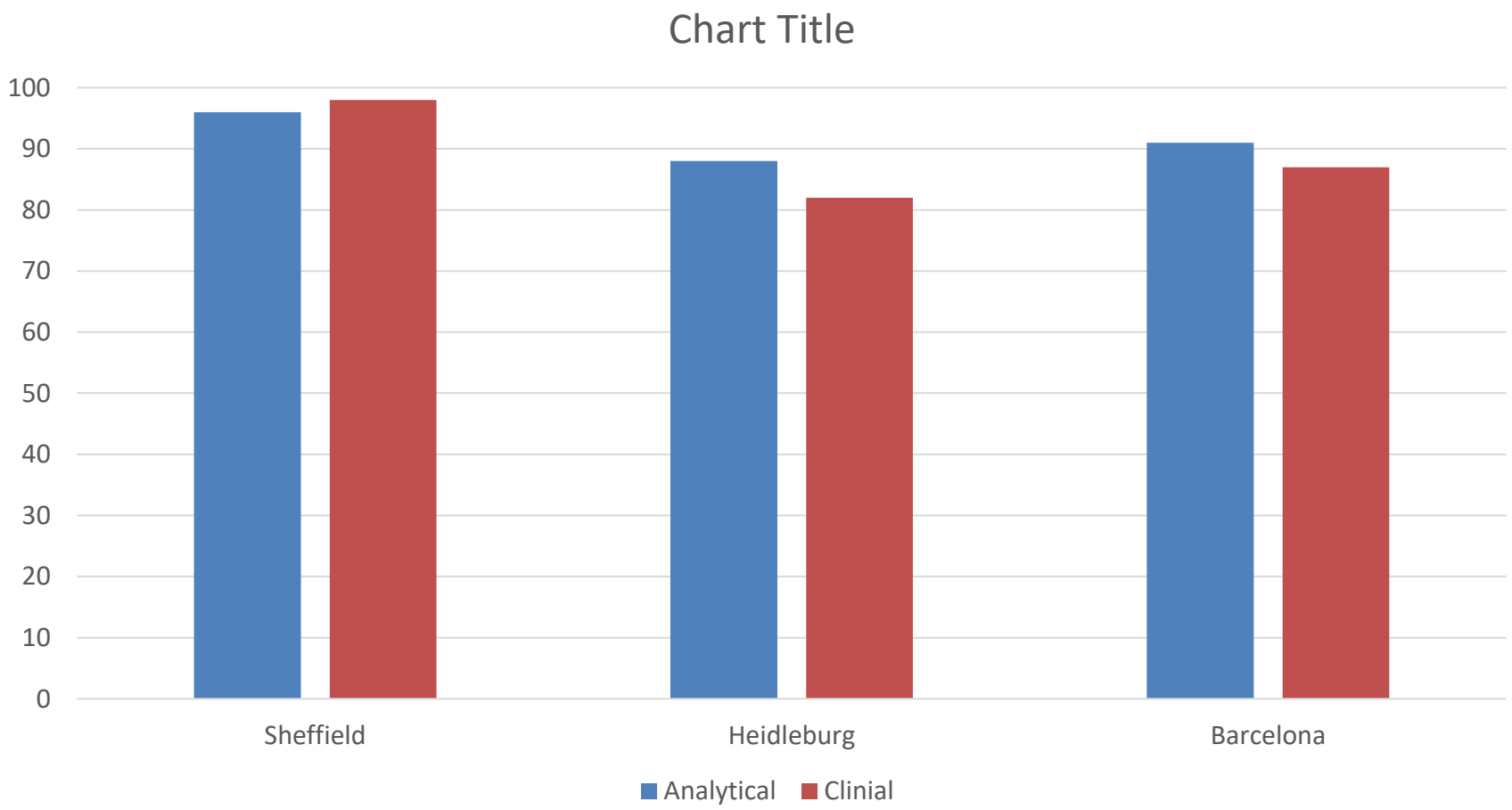
37-year-old man diagnosed with isolated 3 Methylcrotonyl-CoA carboxylase deficiency following family studies

No treatment required and remains well.

Sample B: 3 Methylcrotonyl CoA carboxylase deficiency

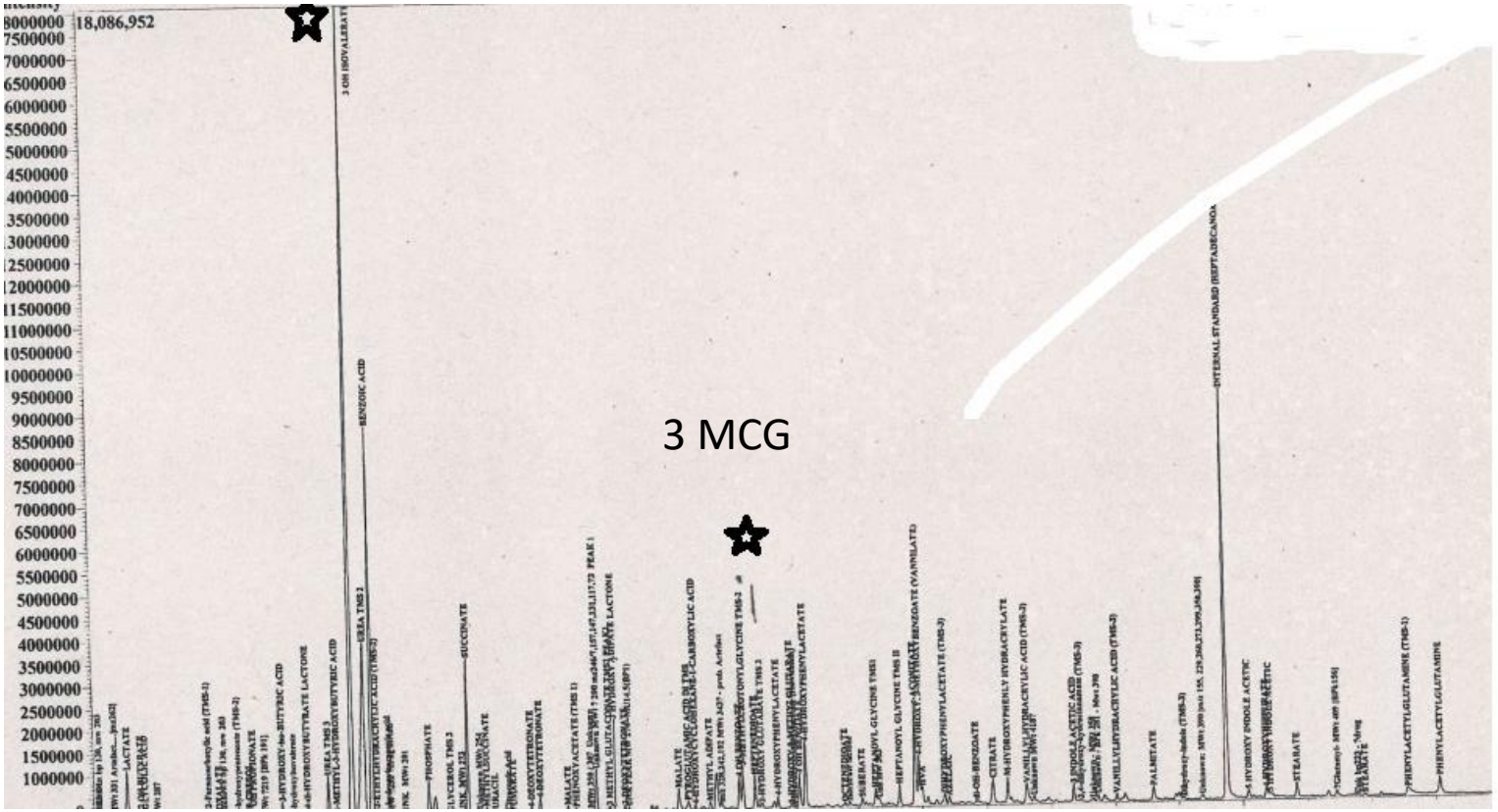
- Common sample sent to all three centres
- **Proficiency:**
  - Sheffield 95 %,
  - Heidelberg 82 %
  - Barcelona (89 %)
- **Scoring agreed across all three centres:**
  - 3Methylcrotonyl Glycine = 1pt
  - 3 Hydroxy isovaleric acidI = 1pt
  - 3Methylcrotonyl CoA Carboxylase deficiency = 2pts
  - Biotinidase deficiency/HCS = 1pt only

Sample B – common sample



Chromatogram

3 OH IVA



Discussion

- Heidelberg scheme – 10 labs scored zero for **interpretation**.
- Sheffield scheme – 2 labs scored zero for **interpretation**.
- Participants who scored zero typically only identified increased 3 hydroxy isovaleric acid and did not identify the other metabolites which would lead to a benign disorder.
- As this is a ‘benign’ disorder no critical errors will be applied.

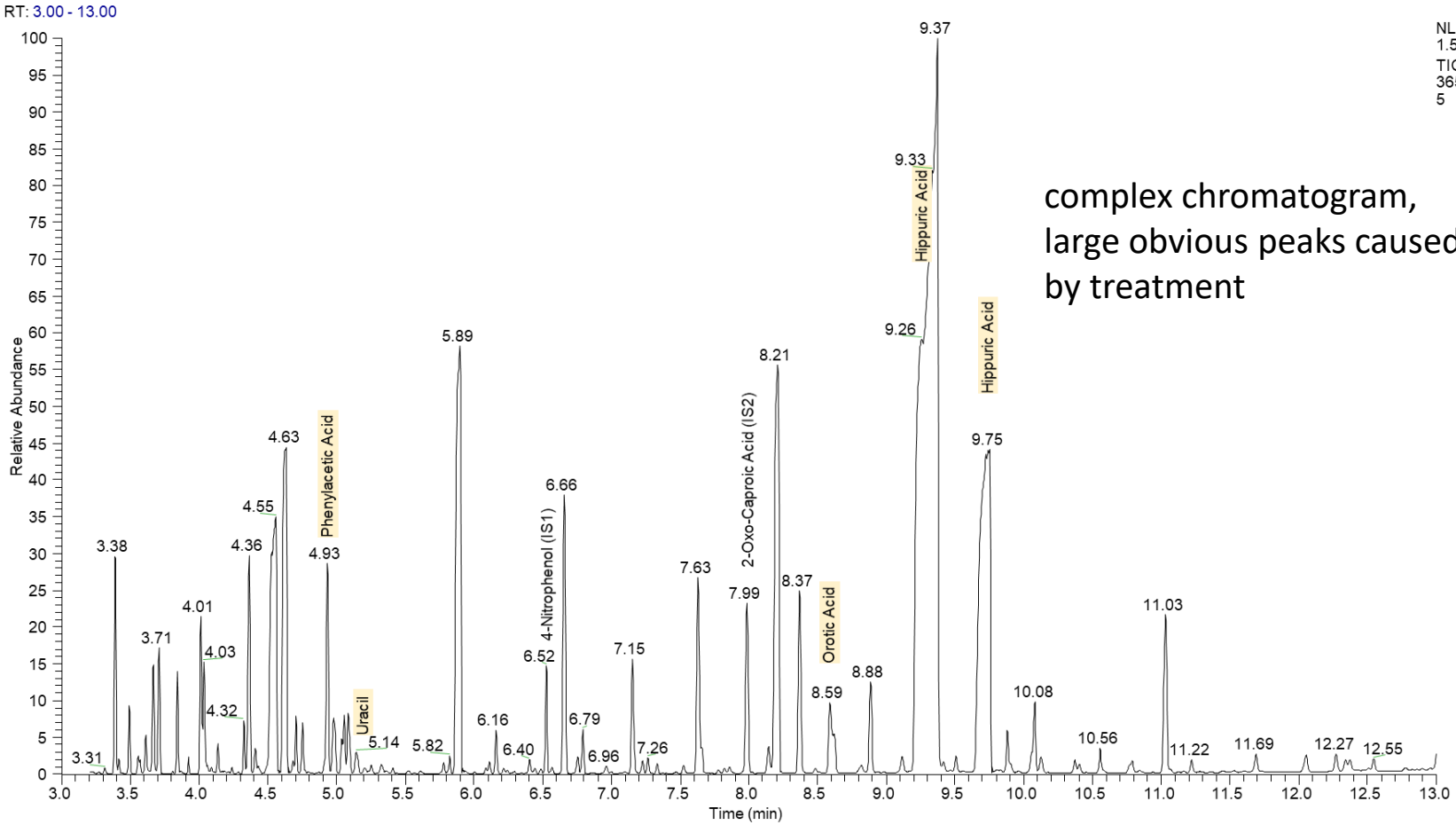
Proficiency across all the samples Heidelberg Scheme 2024:

- **Aromatic L-amino decarboxylase (AADC) deficiency-** 64%
- **Isovaleric aciduria-** 99%
- **Ornithine transcarbamylase deficiency-** 73% (Barcelona 76%)
- **Alkaptonuria-** 94%
- **Normal sample-** 95%
- **Methylmalonic Aciduria cblB type-** 94%

Difficult samples:
Sample C 2024 (HD): OTC deficiency

- Clinical details provided to participants: QLOU-DH-2024-C **10-year-old girl with spina bifida, Arnold-Chiari malformation and severe psychomotor retardation.**
- Patient had surgical interventions but no signs of hyperammonemic decompensations.
- Metabolic examinations due to neurological deterioration revealed hyperammonemia
- Sample was taken was taken while on scavenger therapy with sodium benzoate, sodium phenylbutyrate and L-citrulline

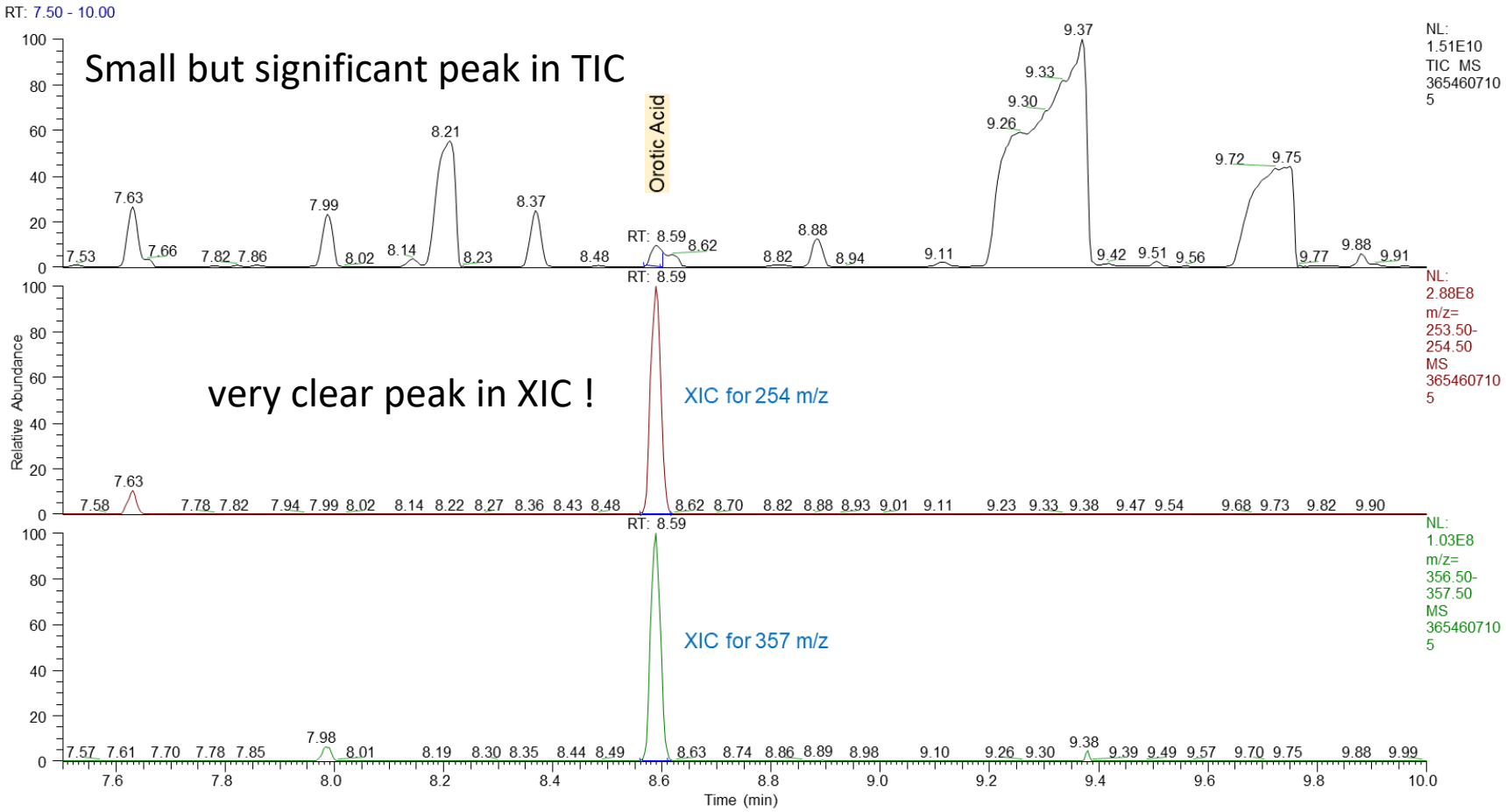
Difficult samples
HD - 2024 C / proficiency 69%



complex chromatogram, large obvious peaks caused by treatment

Difficult samples

HD - 2024 C – key metabolite: orotic acid



Proficiency across all the samples Barcelona Scheme 2024:

- **Phenylketonuria**- 99%
- **L-2-hydroxyglutaric aciduria-** 92 %
- **Ornithine transcarbamylase deficiency-** 76%
- **Multiple acyl-CoA dehydrogenase deficiency-** 85%
- **Normal sample-** 94%
- **Glutaric aciduria type I**- 95%

Sample C 2024 (EB): OTC

- **Clinical information:** **Female** with development delay, diagnosed at 6 years old when she presented **hiperamonemia after an episode of infection**. At present she is **under treatment**. Patient Age 31 years old

Sample C 2024 (EB): OTC deficiency

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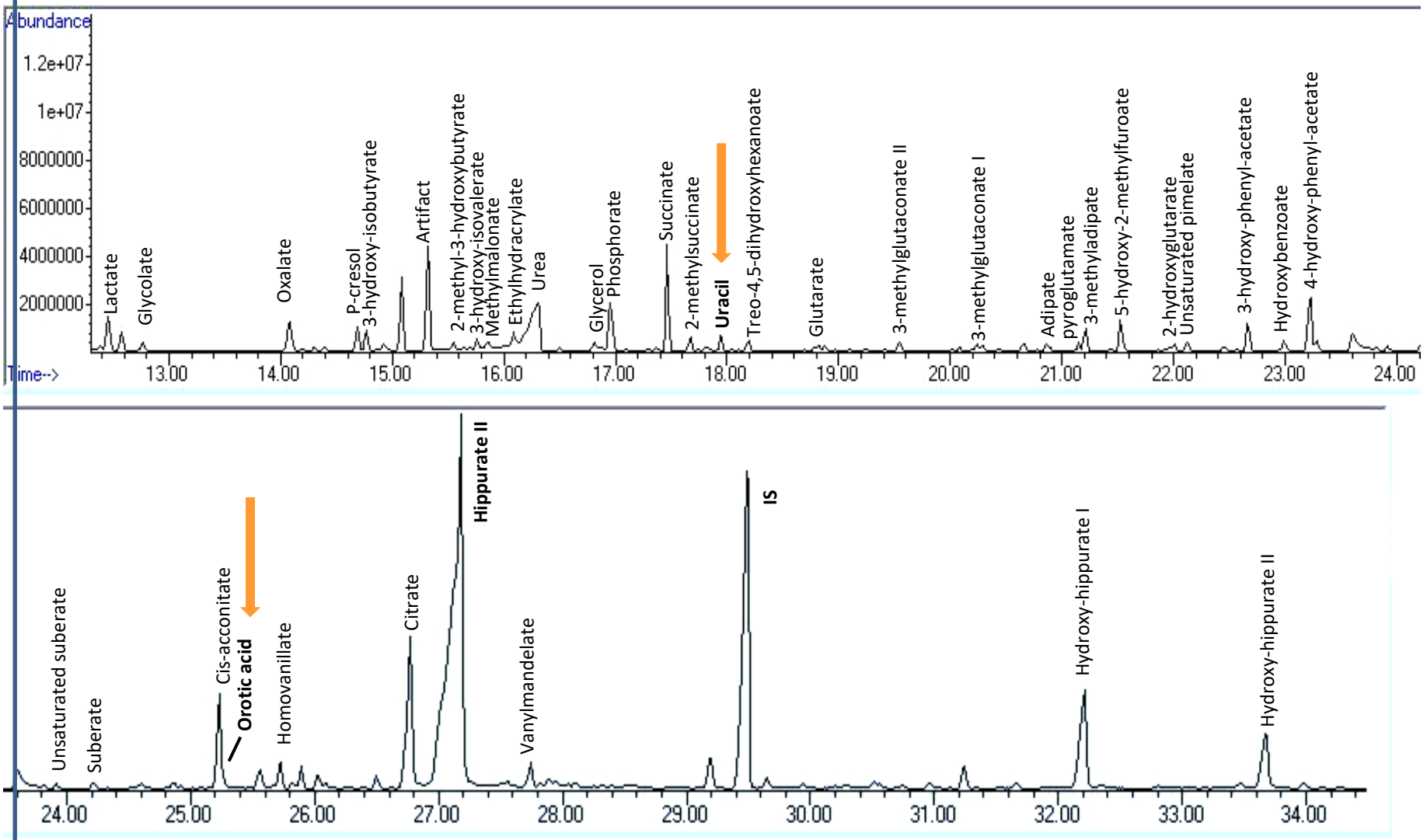
TIC QLOU-EB-2024-C



Sample C 2024 (EB): OTC deficiency

- **Analytical performance**

-**52%** reported correctly the increase of **uracil**, **one of them put the increase of uracil in the comment box** and not in the key metabolite box.
-**40%** reported the increase of **orotic acid**, **six of them put the increase of orotic acid in the comment box** and not in the key metabolite box.
-**21%** reported increased **hippurate**.

SCORE:
- 2 points: detection of uracil and /or orotic acid.
- 1 point: detection of increased hippurate without any key metabolite.

With the clinical information an urea cycle disorder should be suspected, therefore **it is better to extract ions of the key metabolites** to increase the sensitivity

Sample C 2024 (EB): OTC deficiency

- **Interpretative proficiency**

-**54%** reported correctly the diagnosis of **OTC or urea cycle disorder**.
-**11** participants reported as **normal profile**, and **one lab not put anaything in diagnosis box.**

SCORE:
-2 points are given for the diagnosis or alternative diagnosis of OTC or urea cycle disorder.
-1 point is given for the recommendations to analyze amino acids, orotic acid o rule out urea cycle disorder.

- **Recommendations**

The majority of the laboratories recommend performing aminoacids in plasma and in urine, orotic acid in urine and molecular diagnosis of urea cycle disorders.

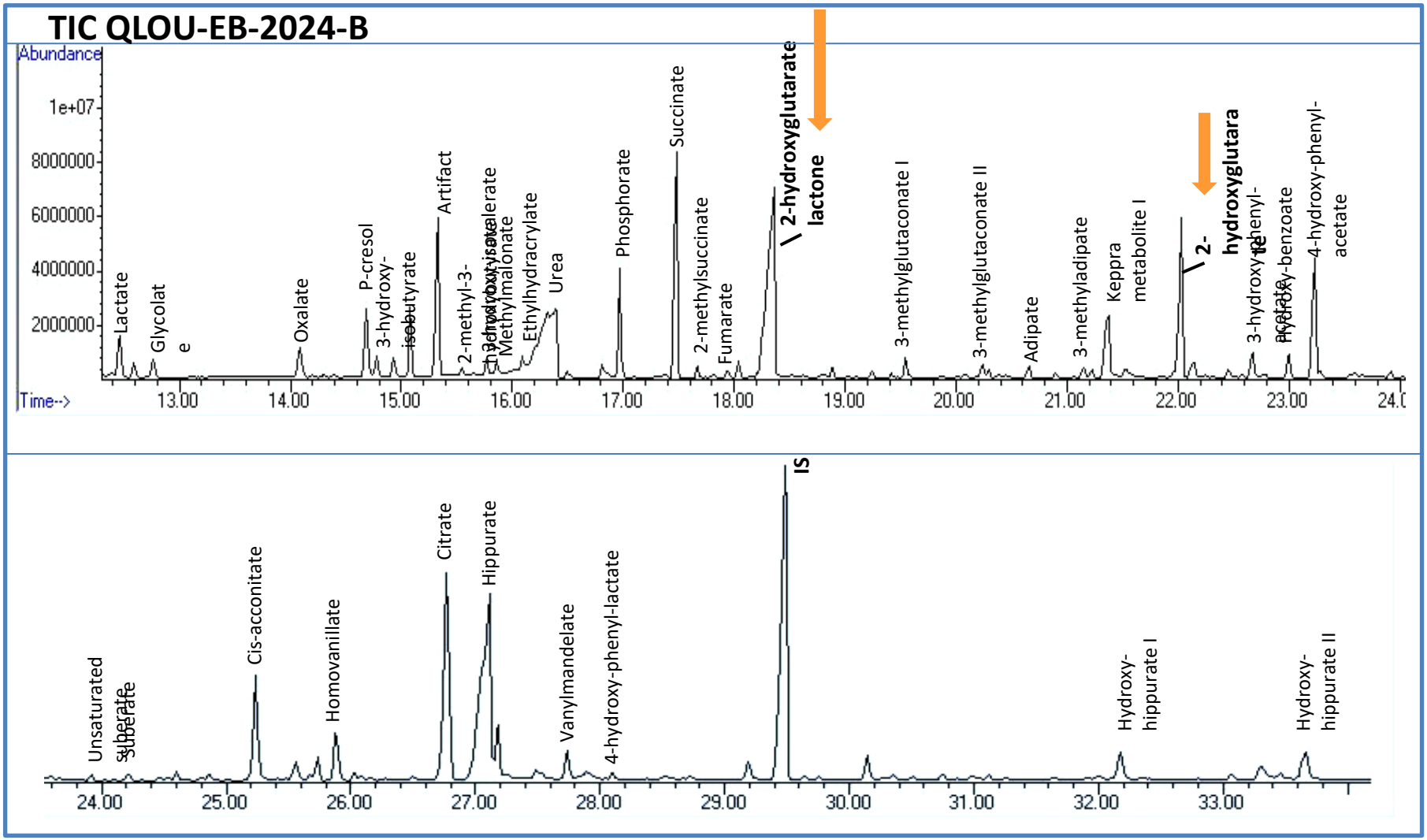
Proficiency across all the samples Barcelona Scheme 2024:

- **Phenylketonuria**- 99%
- **L-2-hydroxyglutaric aciduria-** 92 %
- **Ornithine transcarbamylase deficiency-** 76%
- **Multiple acyl-CoA dehydrogenase deficiency-** 85%
- **Normal sample-** 94%
- **Glutaric aciduria type I**- 95%

Sample B 2024 (EB): L-2-hydroxyglutaric aciduria

Clinical information: Patient diagnosed in childhood. Currently **undergoing treatment** and has mental retardation, **dystonic tetraparesis, and microcephaly**. Patient Age 19 years old.

Sample B 2024 (EB): L-2-hydroxyglutaric aciduria



Organic acids method:

-Extraction twice with ethylacetate without oximation. TMS-derivatization

-GC-MS with 60 m x 0.25 mm ID HP-5MS capillary column.

Sample B 2024 (EB): L-2-hydroxyglutaric aciduria

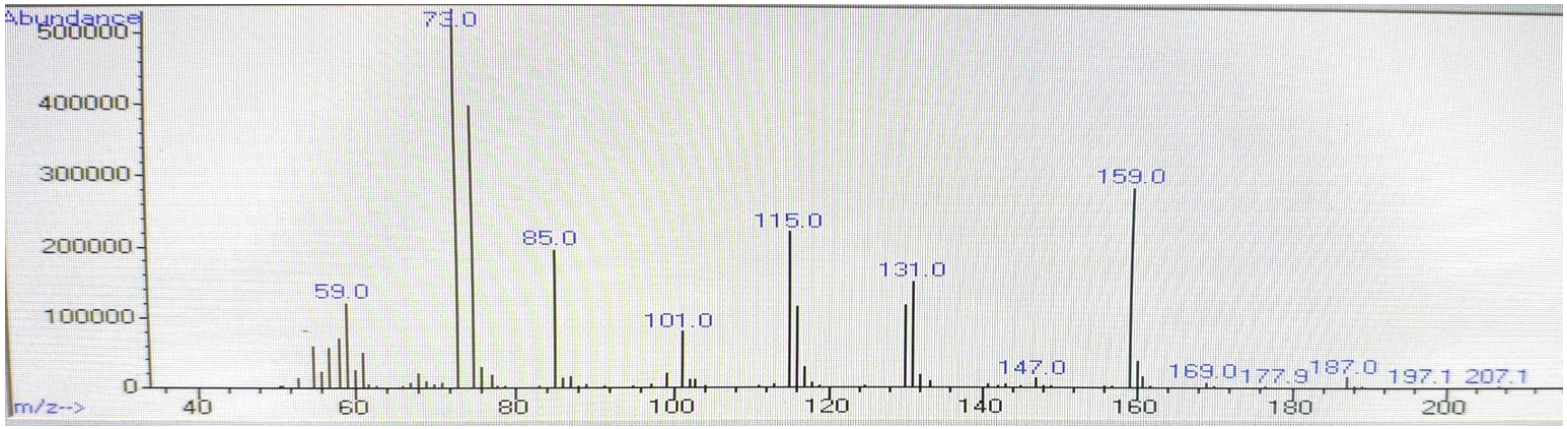
- **Analytical performance**

-**96%** reported correctly the increase of **2-hydroxyglutarate**. One of them put the increase of this metabolite in comment box.
-**55%** also reported the increase of **2-hydroxyglutarate lactone**

SCORE: 2 points are given for the detection of 2-hydroxyglutarate.

Important to detect

Mass spectrum 2-hydroxyglutarate lactone



Sample B 2024 (EB): L-2-hydroxyglutaric aciduria

- **Interpretative proficiency**

-**84%** reported the **L-2-hydroxyglutaric aciduria** as correct diagnosis.
-**Two labs** diagnosed as **combined D- and L-2-hydroxyglutaric aciduria**, **without** the correct **further studies** to rule out other types of 2-hydroxyglutaric acidurias.
-**One lab** reported as **D-2-hydroxyglutaric aciduria due to the chiral study**, but the diagnosis was L-2-hydroxuglutric aciduria, and **another two labs** put **2-hydroxyglutaric aciduria as alternative but without** specific further **recommendations**.
-**Three labs** reported multyple acil-CoA dehydrogenase deficiency (**MADD**).
-**Two labs** reported as **normal or other** not related alternative **disorders**.

SCORE:
- 2 points: the diagnosis of L-2-hydroxiglutaric aciduria or 2-hydroxyglutaric aciduria with the recommendation to study chiral study or differential diagnosis by molecular studies to differentiate D- and L-2- Hydroxiglutarica aciduria.
- 1 point: the diagnosis of D-2-hydroxyglutaric acid and 2-hydroxiglutaric aciduria without additional specific recommendations.

Sample B 2024 (EB): L-2-hydroxyglutaric aciduria

- **Recommendations**

The majority of the participants recommend:
-Chiral metabolite analysis to distinguish L/D forms.
-Perform genetic analysis of *L2HGDH* gene and *D2HGDH*, less laboratories mentioned the analysis of *IDH2* and *SLC25A1* genes.

CRITICAL ERRORS:
-2 labs that reported as normal sample
-One lab that gave the diagnosis of Maple syrup urine disease (MSUD).

Sample D 2024 (EB): MADD

Clinical information: Female diagnosed at 4 years of age. She presented **hypoglycemia and hiperamoniemia after pneumonia**. At present she is **under treatment**. Patient age 21 years old

Sample D 2024 (EB): MADD

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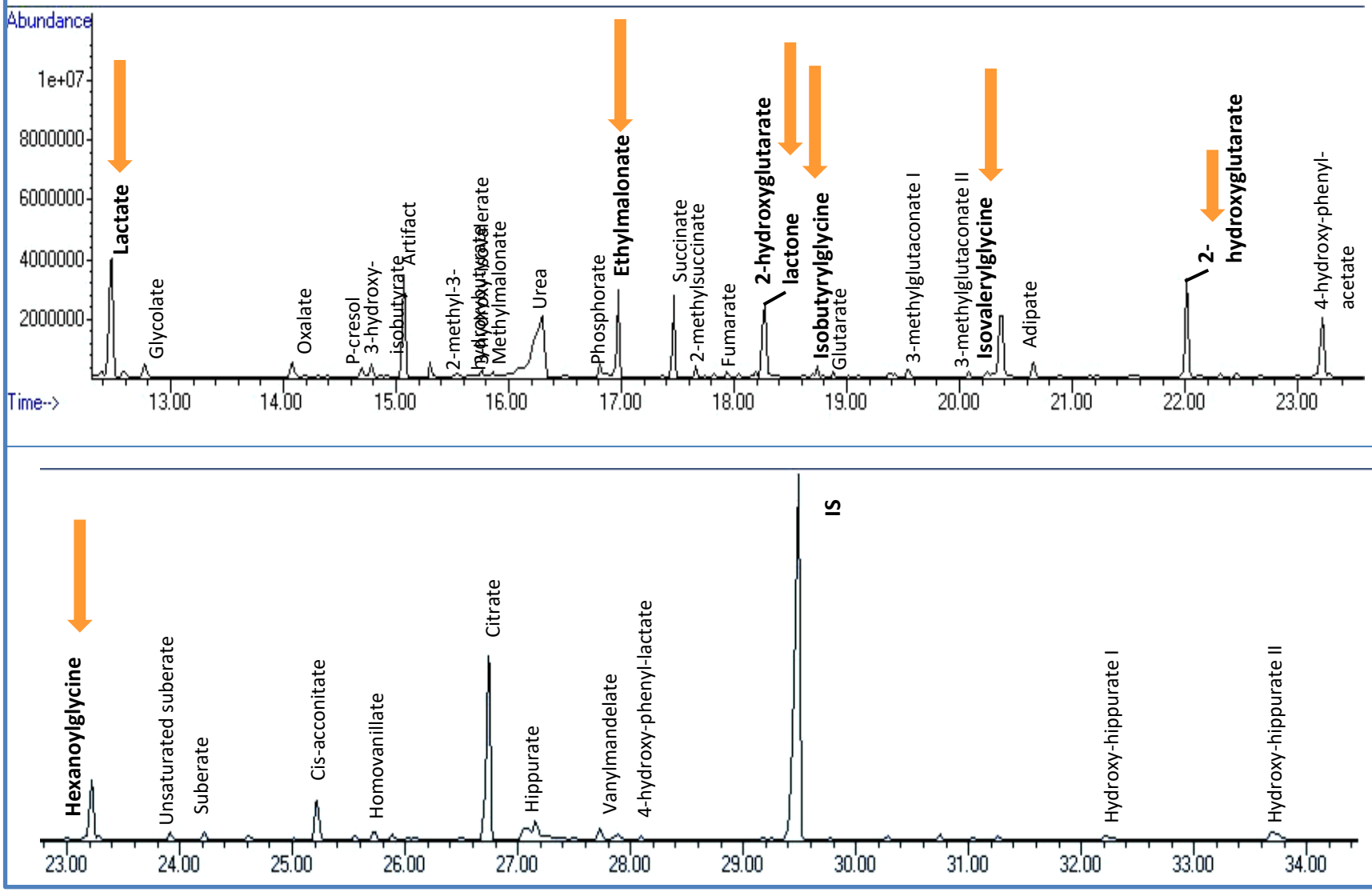
TIC QLOU-EB-2024-D



Sample D 2024 (EB): MADD

- **Analytical performance**

-72% detected the increase of **ethylmalonate**
-94% detected increase of **2-hydroxyglutarate**
-38% detected **2-hydroxyglutarate lactone**
-55% reported increased **isovalerylglycine**
-46% the increase of **hexanoylglycine**.
-52% the increase of **lactate**
The increase of other acylglycines (**isobutyrylglycine and suberylglycine**), **and other dicarboxylic acids were detected between the 20 and 14%** of participants.

SCORE:
-2 points: the detection of 2-hydroxyglutarate and ethylmalonate or some acylglicines or dicarboxylic acids.
-1 point: only the increase of one key metabolite is given.

Sample D 2024 (EB): MADD

- **Interpretative proficiency**

-**80%** reported **MAD deficiency** or glutaric aciduria type II as the correct diagnosis.
-**9** participants give the diagnosis of **2-hydroxylutaric aciduria**.
-1 laboratory reported propionic acidemia, other multyple mitochondrial syndrome 1, other isovaleric acidemia and other hyperinsulinism.
- **1 lab reported as normal** sample **apparently due to a swap** with sample E.

SCORE:
-2 points: the diagnosis of MAD deficiency or glutaric aciduria type II.
-1 point: other diagnosis is reported but in the recommendations the study of acylcarnitines is specified.

Sample D 2024 (EB): MADD

- **Recommendations**

-The majority of laboratories recommended to perform acylcarnitine analysis and genetic analysis, the genes that should be included: *ETFA, ETFB, ETFDH, FLAD1, SLC25A32, SLC52A1, SLC52A2, SLC52A3.*

CRITICAL ERRORS:

-1 lab that reported as propionic academia

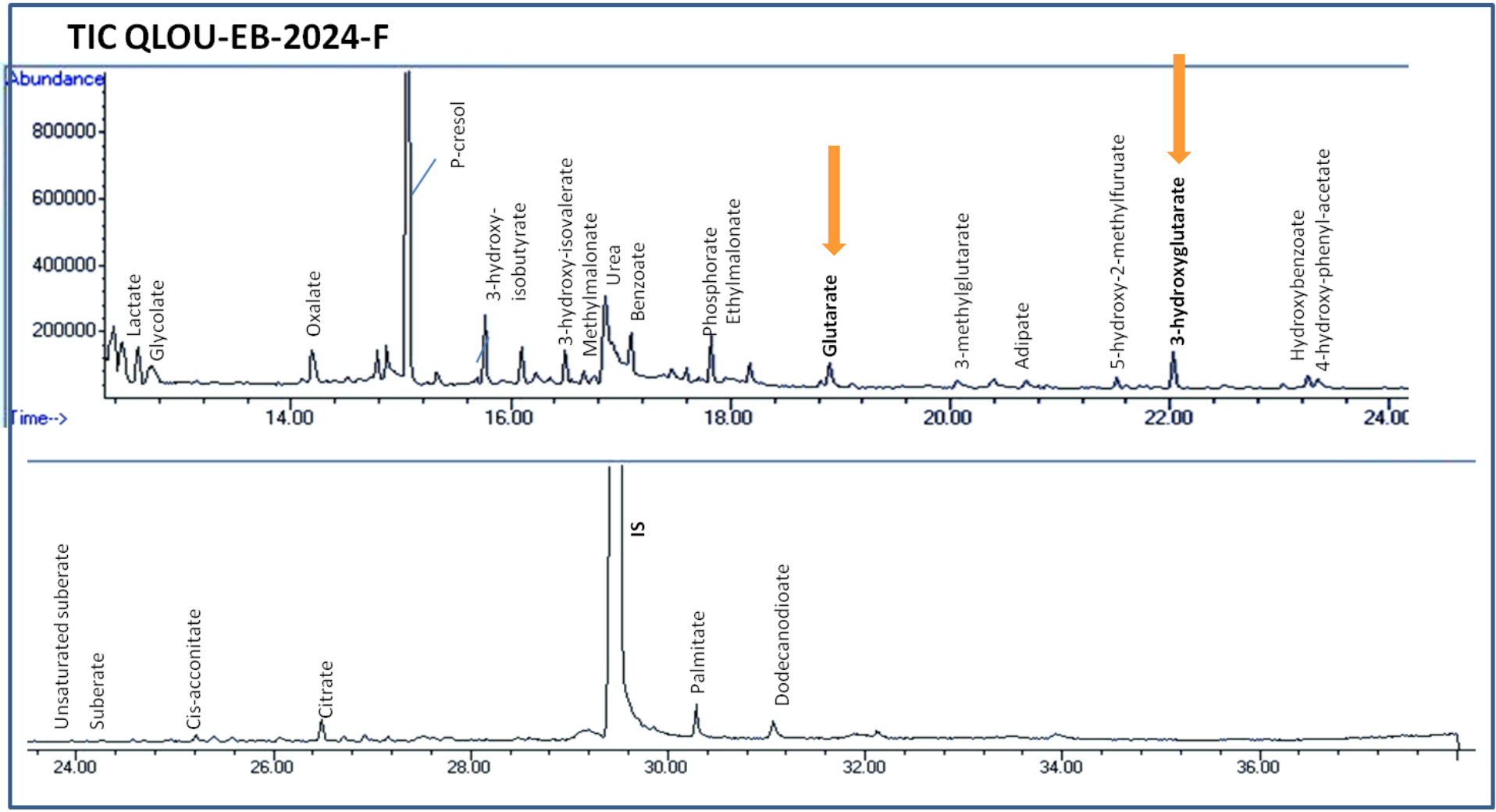
-1 lab that gave the diagnosis of hyperinsulinism

-1 lab that reported as normal apparently due to a swap with sample E, and without additional specific recommendations.

Sample E 2024 (EB): Glutaric aciduria type I

Clinical information: Patient diagnosed at 15 months of age with **movement disorders and psychomotor retardation**. Currently he is **under treatment** and presents with a **tetraparesia**. Patient age 25 years old

Sample E 2024 (EB): Glutaric aciduria type I



Sample E 2024 (EB): Glutaric aciduria type I

- **Analytical performance**

-**92%** detected the increase of **glutarate and 3-hydroxyglutarate**.
-**5 laboratories only** detected **one** of the two metabolites.
-One laboratory only reported the increase of 3-methylglutaconic acid and 3-methylglutaric acid and other detected increase of 2-hydroxyglutarate.

SCORE:
-2 points: the detection of the glutarate and 3-hydroxyglutarate.
-1 point: if only one of the two metabolites is detected.

Sample E 2024 (EB): Glutaric aciduria type I

- **Interpretative proficiency**

-**96%** reported **glutaric aciduria type 1** or glutaryl-CoA dehydrogenase deficiency as correct diagnosis.
-**Other individual reported diagnoses** were: 2-hydroxyglutaric aciduria, lipoic deficiency and 3-methylglutconic aciduria.

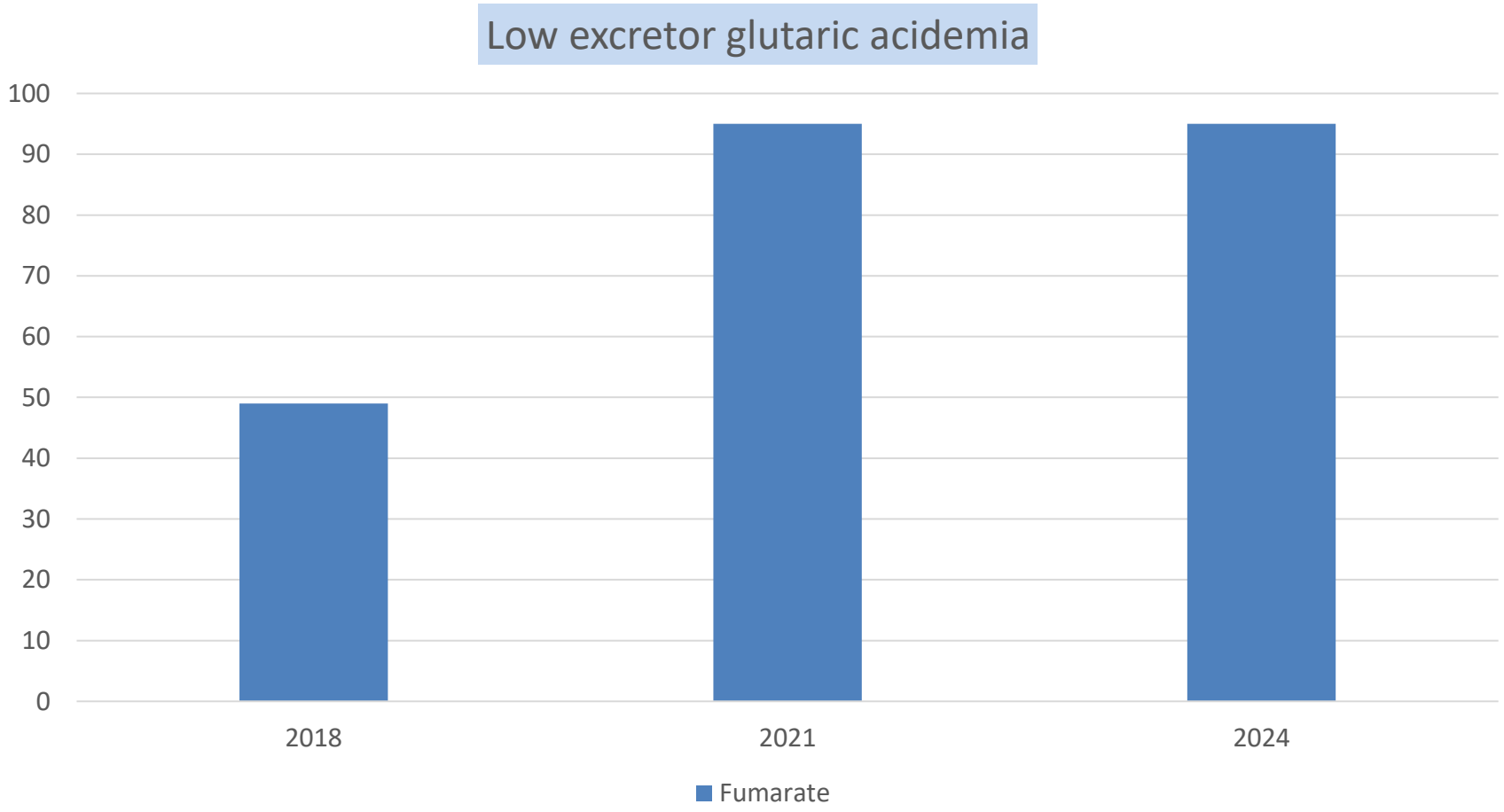
SCORE: 2 points are given for the correct diagnosis of glutaric aciduria type I.

- **Recommendations**

The majority of the participants recommended analysis of acylcarnitines in plasma or DBS or urine, plasma aminoacid measurement and molecular studies of *GCDH* gene.

CRITICAL ERRORS: 1 lab that reported as 2-hydroxyglutaric aciduria, 1 lab that gave the diagnosis of lipoic acid defects and 1 lab that reported the diagnosis of 3-methylglutaconic aciduria.

Proficiency over the years



Take home

✓Filling in the information in the correct boxes is important.

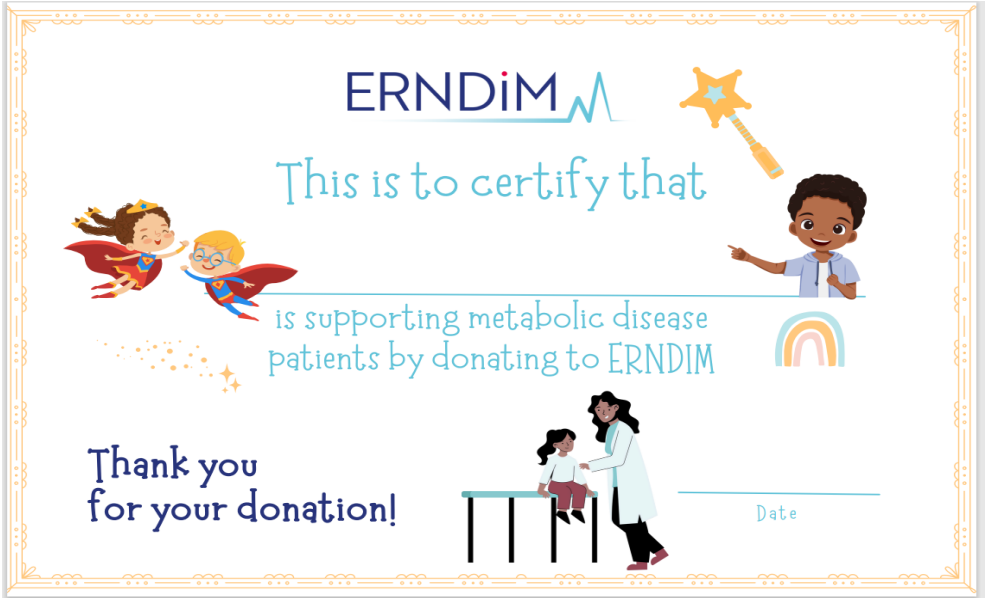
✓The clinical information is important.

✓Consider including the necessary differential diagnosis process in the recommendations.

✓Read the reports and consult with the scientific advisor about any doubts.

Plea for sample

- We have a limited store of samples and make a plea each year to send any donations. We need approximately 100 to 200 ml of urine.
- Please contact ERNDIM or your SA for a consent form and a certificate for the donor:



ERNDiM

Thank you for your participation in QLOU Schemes and for attending the workshop