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Published: 14 March 2023[1]

Qualitative Organic Acids

Centre: United Kingdom

Final Report 2022

prepared by
Mrs C Scott and Miss S Colyer

Note: This annual report is intended for participants of the ERNDIM QLOU Sheffield scheme. The contents should not be used for any publication without permission of the Scientific Advisor.

The fact that your laboratory participates in ERNDIM schemes is not confidential, however, the raw data and performance scores are confidential and will only be shared within ERNDIM for the purpose of evaluating your laboratories performance, unless ERNDIM is required to disclose performance data by a relevant government agency. For details please see the ERNDIM Privacy Policy on www.erndim.org.

In 2022, 74 labs participated to the Qualitative Organic Acid Testing Scheme Sheffield.

1. Geographical distribution of participants

For the first survey, 73 and second survey 70 laboratories submitted results.

Country	Number of participants
Australia	6
Belgium	7
Finland	2
Germany	1
Hungary	1
Ireland	1
Israel	3
Japan	6
Malaysia	3
New Zealand	1
Norway	1

[1] If this report is not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document.

Country	Number of participants
Pakistan	1
Poland	2
Slovakia	2
South Africa	2
Sweden	2
United Kingdom	16
United States of America	17

2. Design and logistics of the scheme including sample information

The scheme has been designed and planned by Camilla Scott as Scientific Advisor and coordinated by Alessandro Salemma scheme organiser (sub-contractor on behalf of CSCQ), both appointed by and according to procedures laid down the ERNDIM Board.
CSCQ dispatches QLOU EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports. Existing QLOU scheme participants can log on to the CSCQ results submission website at:
https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php

2 surveys	Round 1: patients A, B and C
	Round 2: patients D, E and F

Origin of patients: all urine samples have been provided by the scheme organizers.

Patient A:staff donation
Patient B:donated from Rotterdam Dr Ruijter
Patient C:historical patient donation
Patient D:historical patient donation
Patient E:historical patient donation
Patient F:historical patient donation

The samples have been heat-treated. They were pre-analysed in our institute after 3 days incubation at ambient temperature (to mimic possible changes that might arise during transport). In all six samples the typical metabolic profiles were preserved after this process.
Mailing: samples were sent by DHL; FedEx or the Swiss Post at room temperature.

3. Tests

Analyses of qualitative organic acids.

4. Schedule of the scheme

- Feb 2, 2022: shipment of samples by CSCQ
- May 9, 2022: analysis start and website submission 1st round (A-C)
- May 30, 2022: results submission deadline
- Sep 26, 2022: deadline for result submission (Survey 2)
- July 11, 2022: report of Survey 1 by e-mail
- October, 2022: report of Survey 2 by e-mail
- January 20, 2023: annual report with scoring.

5. Results

70 out of 74 labs returned results for both surveys

	Survey 1	Survey 2
Receipt of results	73	70
No answer	1	4

6. Web site reporting

The website reporting system is compulsory for all centres. Please read carefully the following advice:

- Results
  - Give quantitative data as much as possible.
  - Enter the key metabolites with the evaluation **in the tables.**
  - If the profile is normal: enter “Normal profile” in “Key metabolites”.
  - **Don’t enter results in the “comments” window**, **otherwise your results will not be included in the evaluation program.**
- Recommendations = **advice for further investigation**.
  - Scored together with the interpretative score.
  - Advice for treatment are not scored but may be used in the overall assessment.
  - **Don’t give advice for further investigation in “Comments on diagnosis”**: it will not be included in the evaluation program.

7. Scoring and evaluation of results

Information regarding procedures for establishment of assigned values, statistical analysis, interpretation of statistical analysis etc. can be found in generic documents on the ERNDIM website.
The scoring system has been established by the International Scientific Advisory Board of ERNDIM. Two criteria are evaluated: 1) analytical performance, 2) interpretative proficiency also considering recommendations for further investigations.

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory or misleading	0
I	Interpretative proficiency & Recommendations	Good (diagnosis was established)	2
		Helpful but incomplete	1
		Misleading or wrong diagnosis	0

The total score is calculated as a sum of these two criteria. The maximum to be achieved is 4 points per sample. The scores were calculated only for laboratories submitting results.

Scoring and certificate of participation: scoring is carried by a second assessor who changes every year as well as by the scientific advisor. The results of QLOU US have also been scored by Dr Judit Garcia Villoria for QLOU Barcelona. At the SAB meeting in November 2022 the definitive scores have been finalised. The concept of critical error was introduced in 2014. A critical error is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient. Thus labs failing to make a correct diagnosis of a sample considered as eligible for this category will be deemed not to have reached a satisfactory performance even if their total points for the year exceed the limit set at the SAB. For 2022, the SAB decided that critical error would be awarded for sample C (Citrullinaemia Type 1) if the diagnosis was missed and/or there were no suggestions for appropriate tests that would ultimately reach the correct diagnosis.

A certificate of participation will be issued for participation and it will be additionally notified whether the participant has received a performance support letter. This performance support letter is sent out if the performance is evaluated as unsatisfactory. One performance support letter will be sent by the Scheme Advisor for 2022. One critical error letter will be sent out for this scheme for 2022. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

At least 17 points from the maximum of 24 (70%) is required for satisfactory performance. Sample B was assessed to be an educational sample and subsequently this score will not be included in the evaluation and the maximum points will be adjusted accordingly to reflect this for performance certification.

8. Results of samples and evaluation of reporting

8.1. Patient A

No significant abnormality.

Patient details provided to participants
Autistic spectrum disorder

Patient details
Sample A was donated from a healthy control.

Analytical performance
Number of labs:

No abnormal organic acids	71
Methylmalonic acid	1
3-hydroxy isovaleric acid	1

Diagnosis / Interpretative proficiency
Number of labs:

No significant abnormality	72
Methylmalonic aciduria	1

Recommendations
No further recommendations.

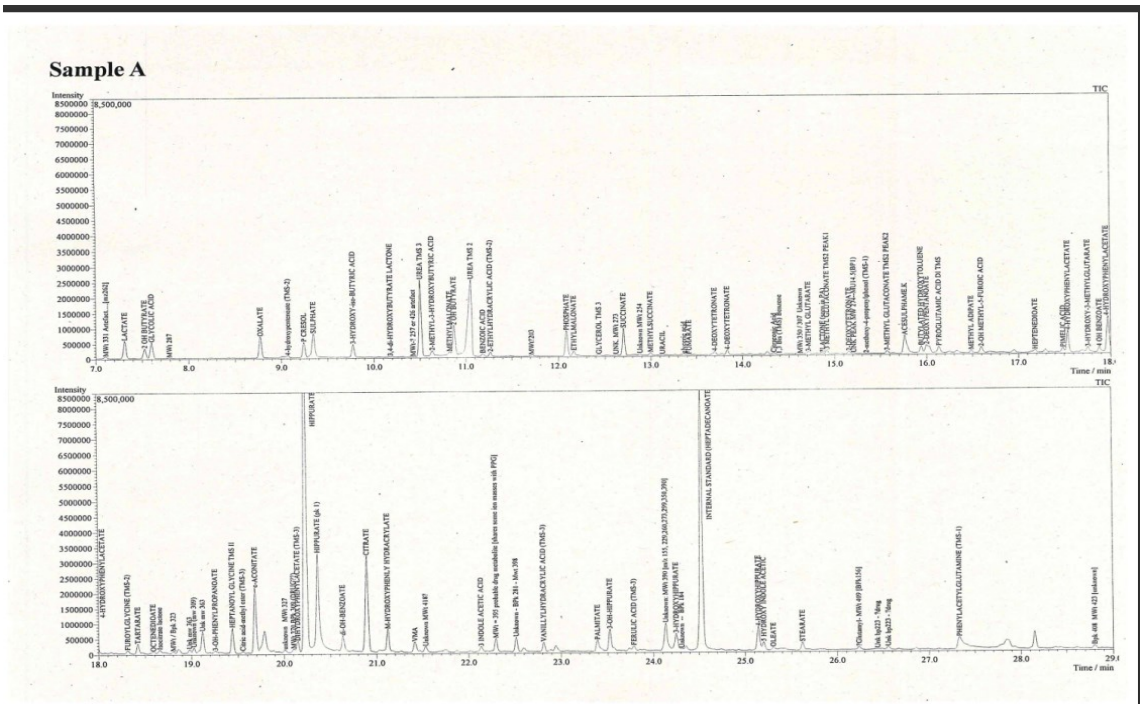
Scoring
Analytical

- No significant abnormality (two points)

Interpretation

- No significant abnormality (two points)

Overall impression
Diagnostic proficiency for this sample was 99 percent. The chromatogram for Sample A:



8.2. Patient B

Aminoacylase 1 deficiency on treatment with L-DOPA.

Patient details provided to participants
Childhood-onset dystonia, diagnosed in adulthood (on treatment)

Patient details
This sample donated from a patient with aminoacylase 1 deficiency on L-dopa treatment.

Analytical performance
Number of labs:

Acetylated amino acids	37
HVA	73

Diagnosis / Interpretative proficiency
Number of labs:

Aminoacylase 1 deficiency	27
AADC	27

Recommendations
Genetic analysis of *ACY1* gene.

Scoring
Analytical
Reported as number of labs:

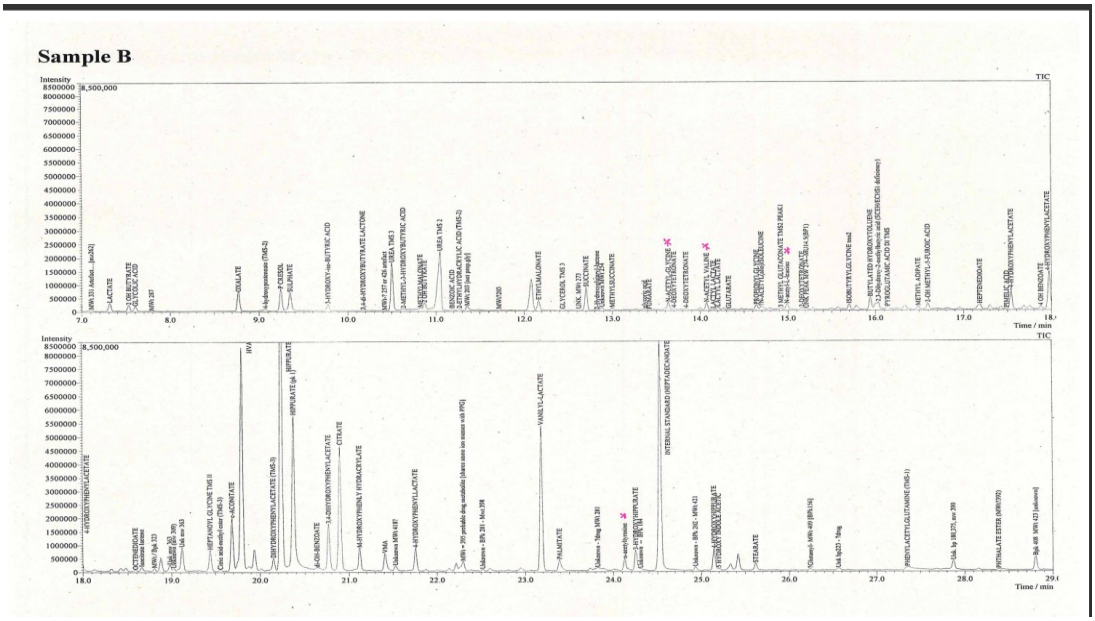
- N-acetylated amino acids (1 point)
- HVA, VLA or L-Dopa (1 point)

Interpretation

- Aminoacylase 1 deficiency (2 points)

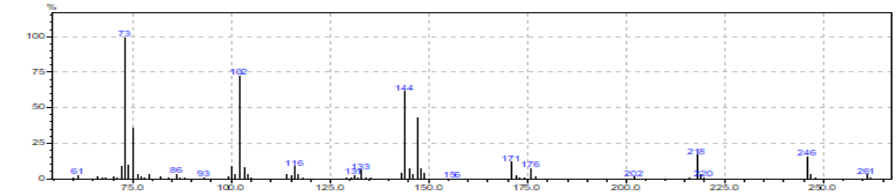
Overall impression
Proficiency for this sample was 58%. It was subsequently agreed at the SAB meeting, in November, that this sample would be deemed educational and the scores would not be counted towards the overall performance of the individual participants.

The chromatogram for sample B:

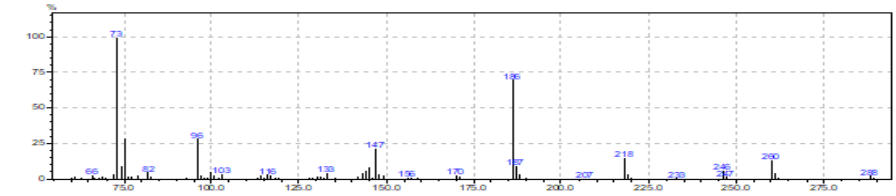


The mass spectrum for the individual acetylated amino acids:

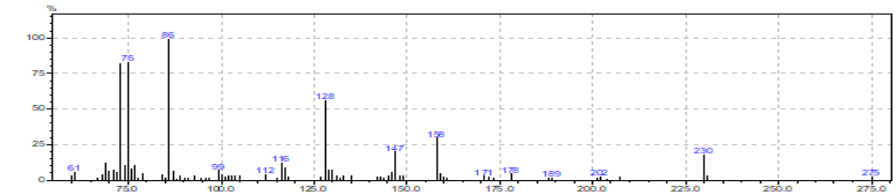
N-acetyl glycine , Ret.Time : [13.633]



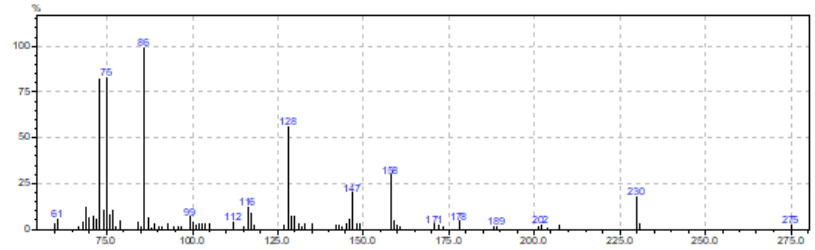
N-acetyl valine, Ret.Time : [14.075]



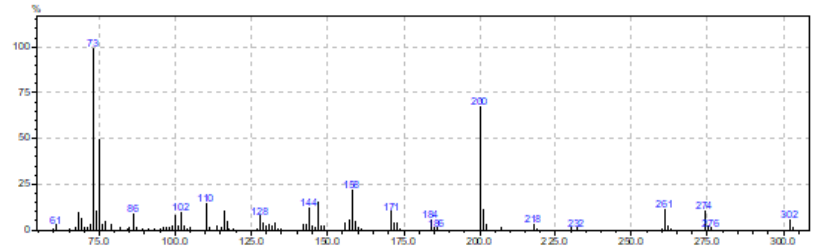
N-acetyl iso-leucine Ret.Time : [14.700]



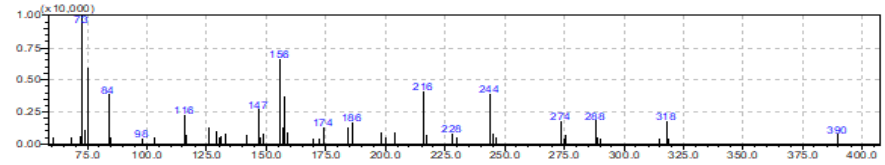
N-acetyl iso-leucine Ret.Time : [14.700]



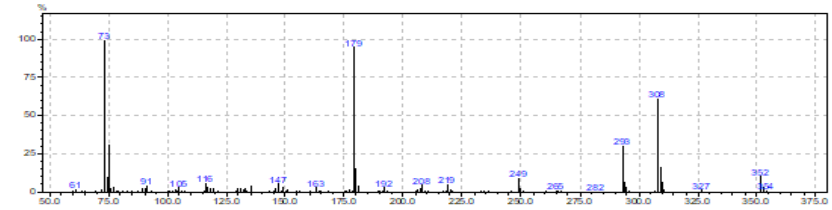
N-acetyl L-leucine, Ret.Time : [14.983]



N-acetylglutamine, Ret.Time : [19.942]



N-acetyltyrosine Ret.Time : [24.133]



8.3. Patient C

Citrullinaemia Type 1 caused by deficient activity of Argnininosuccinate Synthase due to mutations in the ASS gene.

Patient details provided to participants
Hyperammonaemia in the newborn period

Patient details
This sample was donated from a patient with confirmed Citrullinaemia type 1

Analytical performance
Number of labs:

Cyclic derivative of citrulline	46
Orotic acid	68

Diagnosis / Interpretative proficiency
Number of labs:

Citrullinaemia Type 1	46
Urea cycle defect	24

Recommendations
Molecular analysis of the *ASS* gene

Scoring
Analytical

- Cyclic derivative of citrulline (1 point)
- Orotic acid (1 point)

Interpretive

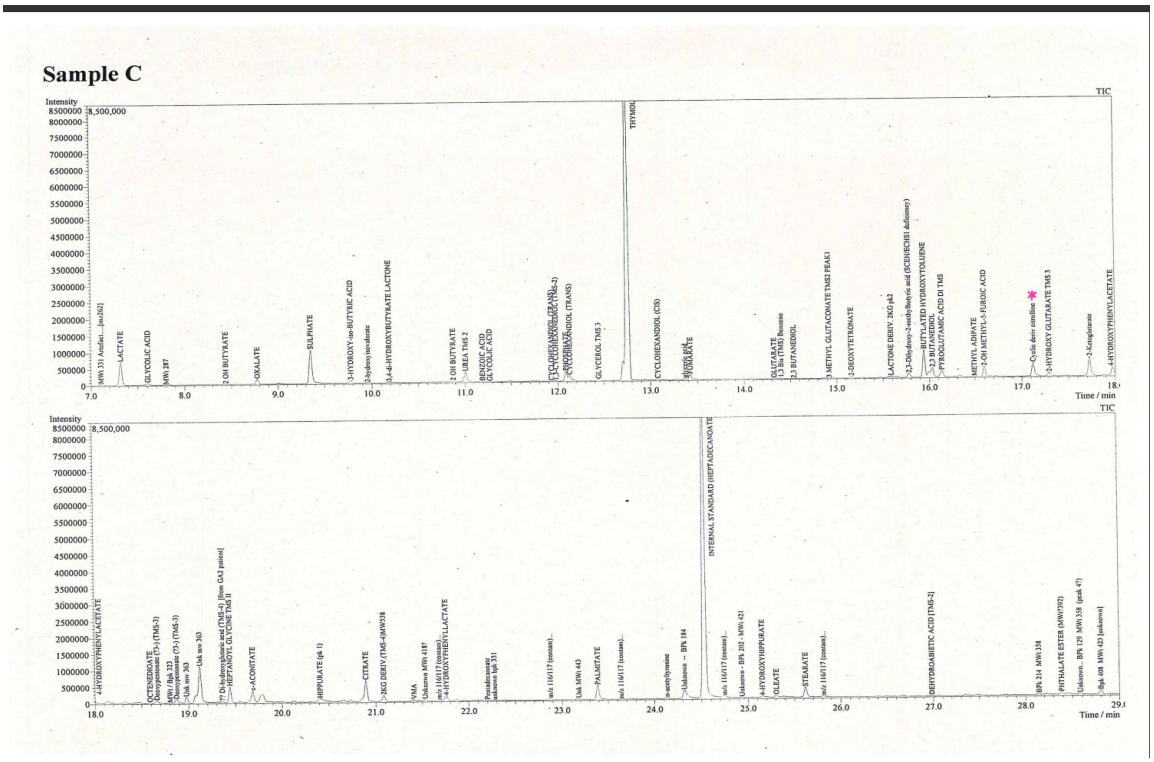
- Citrullinaemia type 1 (2 points)
- Other urea cycle defect (1 point)

Overall impression
Overall proficiency was 80% for this sample. At the recent SAB board in November 2022 it was agreed that if a urea cycle was not considered and/or appropriate follow up tests requested, then this would be deemed a critical error.

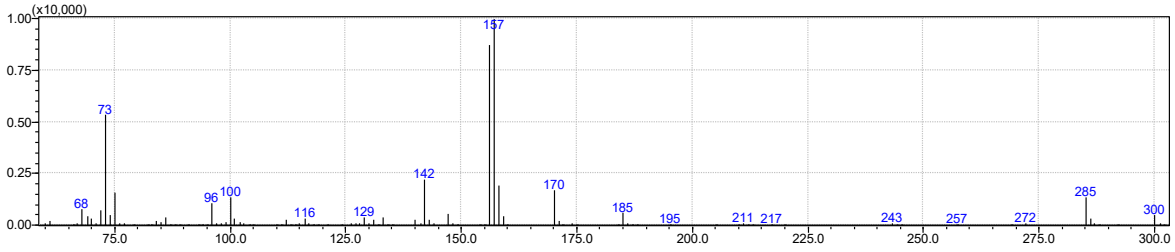
This sample was distributed in 2019 with the following improvement in analytical performance for the cyclic derivative of citrulline but a reduction in performance for the detection of orotic acid:

	2019	**2022**
Cyclic derivative of citrulline	44%	67%
Orotic acid	86%	67%

The chromatogram for sample C:



The mass spectrum for the cyclic derivative of citrulline:



8.4. Patient D

Beta-ketothiolase deficiency a disorder of ketolysis.

Patient details provided to participants
Recurrent vomiting and metabolic acidosis

Patient details
This sample was donated from a patient with confirmed Beta-ketothiolase deficiency.

Analytical performance
Number of labs:

2-methyl-3-hydroxybutyric acid	70
Tiglyglycine	70
2-methyl acetoacetate	25*

*2-methylacetoacetate is an unstable metabolite in urine because it undergoes spontaneous decarboxylation to 2-butanone which is highly volatile. Catanzano F et al. JIMD 2010;33;91.

Diagnosis / Interpretative proficiency
Number of labs:

Beta-ketothiolase	64
2-methyl-CoA mutase deficiency	6

Recommendations
Genetic testing *ACAT1* gene.

Scoring
Analytical

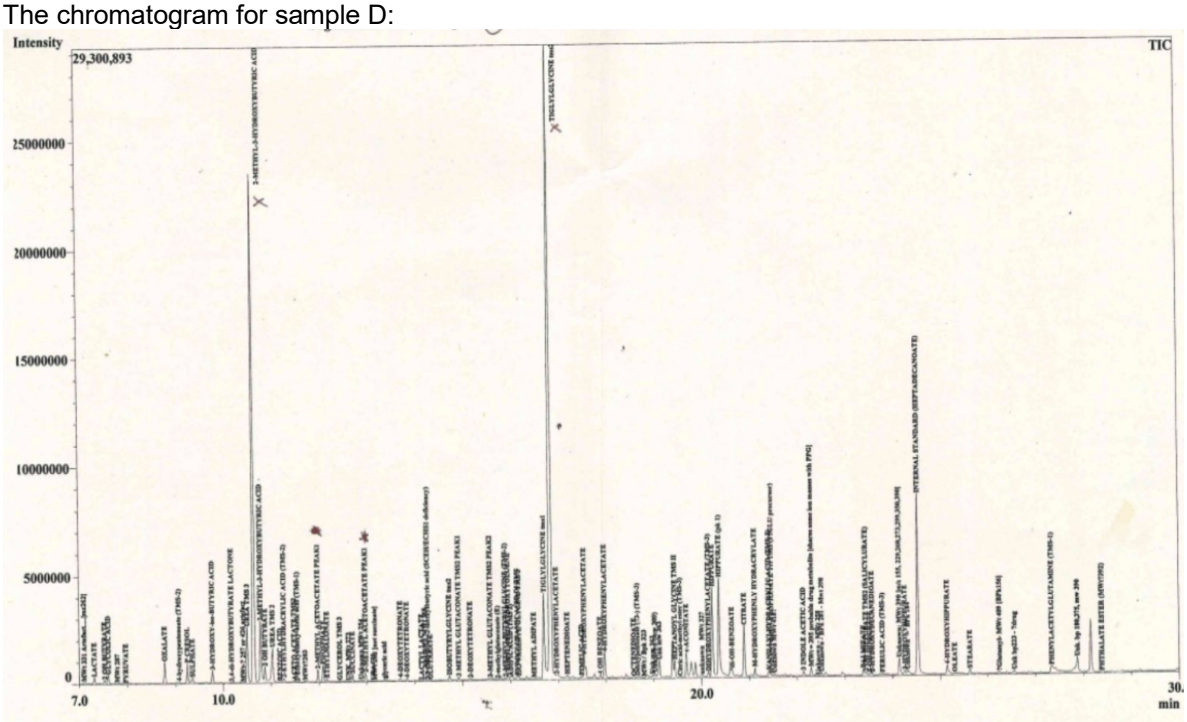
- 2-methyl-3-hydroxybutyic acid (1 point)
- Tiglyglycine (1 point)

Interpretive

- Beta-ketothiolase (as most likely or an alternative diagnosis) (2 points)

Overall impression
Overall proficiency for this sample was very good at 98%.

The chromatogram for sample D:



8.5. Patient E

3-Methylcrotonylglycinuria due to 3-Methylcrotonyl-CoA carboxylase deficiency.

Patient details provided to participants
Mild developmental delay

Patient details
This sample was donated from a patient with confirmed isolated 3-Methylcrotonyl-CoA carboxylase deficiency.

Analytical performance
Number of labs:

3 hydroxyisovaleric acid	70
3-methyl crotonyl glycine	69

Diagnosis / Interpretative proficiency
Number of labs:

3MCC deficiency	67
Biotinidase deficiency	1
Holocarboxylase deficiency	1
Isovaleric acidaemia	1

Recommendations
Genetic analysis of the *MCC1* & *MCC2* genes

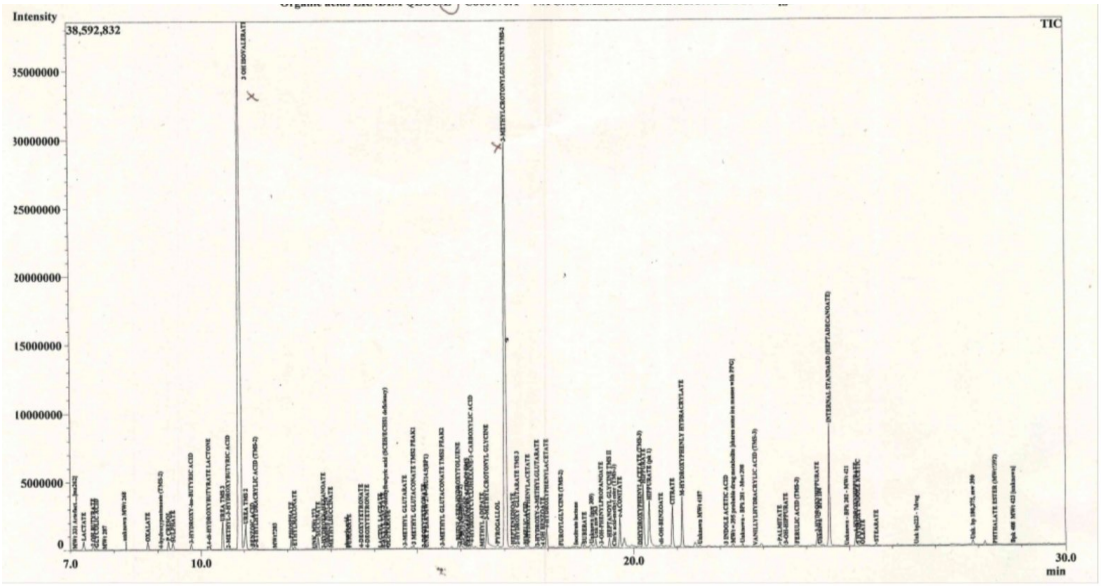
Scoring
Analytical:

- 3 hydroxyisovaleric acid (1 point)
- 3 methyl crotonyl glycine (1 point)

Interpretive

- 3 Methyl-Crotonyl-CoA carboxylase deficiency (2 points)

Overall impression
The proficiency for this sample was 98%. The chromatogram for sample E:



8.6. Patient F

HMG-CoA lyase deficiency (HMGCL gene). A disorder of ketone body metabolism (ketogenesis disorder).

Patient details provided to participants
Profound hypoglycaemia with extreme lethargy

Patient details
This sample was donated from a patient with confirmed HMG-CoA lyase deficiency

Analytical performance
Number of labs:

3-methylglutaconic acid	70
3-hydroxy-3-methylglutaric acid	65

Diagnosis / Interpretative proficiency
Number of labs:

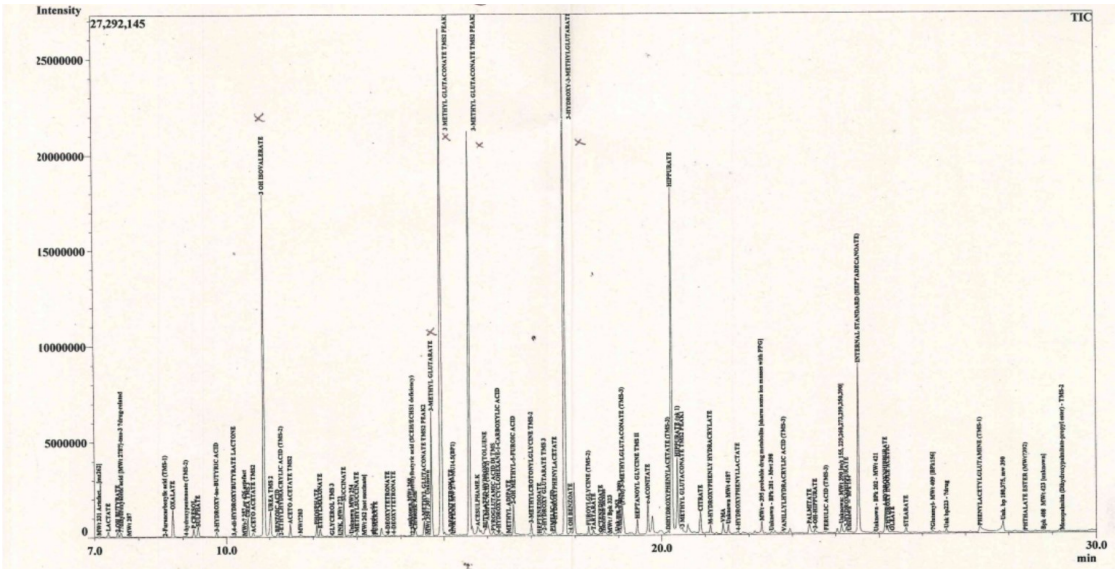
HMG-CoA lyase deficiency	63
3-Methylglutaconyl-CoA hydratase deficiency	7

Recommendations
Molecular analysis of the *HMGCL* gene.

Scoring
Analytical
- 3-hydroxy-3-methylglutaric acid (1 point)
- 3-methylglutaconic acid (1 point)

Interpretation
- HMG CoA lyase deficiency (2 points)

Overall impression
The overall proficiency for sample F was very good at 93%. The chromatogram for sample F:



9. Scores of participants

All data transfer, the submission of data as well as the request and viewing of reports proceed via the QLOU-CSCQ results website. The results of your laboratory are confidential and only accessible to you (with your username and password).The anonymous scores of all laboratories are accessible to all participants and only in your version is your laboratory highlighted in the leftmost column.

Total scores

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)
1	4	1	1	4	4	1	15	62
2	4	2	1	4	4	4	19	79
3	4	1	4	4	4	4	21	88
4	4	2	4	4	4	4	22	92
5	4	1	4	4	4	4	21	88
6	4	4	4	4	4	4	24	100
7	4	1	4	3	4	4	20	83
8	4	2	4	4	4	4	22	92
9	4	4	2	4	4	4	22	92
10	4	2	4	4	4	4	22	92
11	4	1	4	4	4	4	21	88
12	4	4	4	4	4	4	24	100
13	4	1	2	3	4	4	18	75
14	4	4	4	4	4	4	24	100
15	4	4	4	4	4	4	24	100
16	4	1	4	3	2	4	18	75
17	4	4	4	4	4	4	24	100
18	4	4	4	4	4	4	24	100
19	4	1	4	4	4	4	21	88
20	4	1	0	0	0	0	5	21
21	4	1	4	3	4	4	20	83
22	4	1	3	4	1	4	17	71
23	4	4	4	4	4	4	24	100
24	4	1	4	4	4	2	19	79
25	4	4	4	4	4	4	24	100
26	4	2	2	4	4	4	20	83
27	4	1	4	0	0	0	9	38
28	4	4	4	4	4	4	24	100
29	4	1	4	4	4	4	21	88

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)
30	4	4	4	4	4	4	24	100
31	4	2	4	3	4	4	21	88
32	4	4	4	4	4	1	21	88
33	4	2	4	4	4	4	22	92
34	4	4	4	4	4	4	24	100
35	4	2	4	4	4	4	22	92
36	4	2	2	4	4	4	20	83
37	4	4	4	4	4	4	24	100
38	4	1	4	4	4	4	21	88
39	4	4	2	4	4	4	22	92
40	4	1	3	4	4	2	18	75
41	4	1	4	4	4	4	21	88
42	4	1	0	0	0	0	5	21
43	4	4	3	4	4	4	23	96
44	4	1	4	4	4	4	21	88
45	4	4	4	4	4	4	24	100
46	4	1	4	4	4	4	21	88
47	4	1	2	4	4	4	19	79
48	4	2	2	4	4	4	20	83
49	4	1	4	4	4	4	21	88
50	4	1	3	4	4	4	20	83
51	4	1	4	4	4	4	21	88
52	4	4	4	4	4	4	24	100
53	4	4	4	4	4	4	24	100
54	4	4	2	4	4	4	22	92
55	4	1	2	4	4	4	19	79
56	4	1	4	4	4	4	21	88
57	4	4	4	4	4	4	24	100
58	4	1	2	4	4	4	19	79
59	4	4	4	4	4	4	24	100
60	4	4	1	4	4	2	19	79
61	4	1	4	4	4	4	21	88
62	4	2	0	4	4	4	18	75
63	4	4	4	4	4	4	24	100

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)
64	4	2	4	4	4	4	22	92
65	4	1	2	4	4	4	19	79
66	4	1	2	4	4	4	19	79
67	4	2	2	4	4	1	17	71
68	4	4	4	4	4	4	24	100
69	4	4	4	4	4	4	24	100
70	4	1	2	4	4	1	16	67
71	4	4	4	4	4	4	24	100
72	4	2	2	4	4	1	17	71
73	0	0	0	0	0	0	0	0
74	0	1	2	3	2	4	12	50

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 70 % of adequate responses)	68	92
Unsatisfactory performers (< 70 % adequate responses and/or critical error)	2	3
Partial and non-submitters	4	5

Overall Proficiency

Sample ID	Diagnosis	Proficiency (%)	CE
A	Normal	**99**	
B	Aminoacylase 1 deficiency	**58**	
C	Citrullinaemia Type 1	**80**	**1**
D	Beta Ketothiolase	**98**	
E	3 MCC deficiency	**98**	
F	HMG-CoA Lyase deficiency	**93**	

10. Information from the Executive Board and the Scientific Advisory Board

- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 300 ml of urine from a patient affected with an established inborn error of metabolism or “normal” urine, together with a short clinical report.. Each urine sample must be

collected from a single patient (don't send urine spiked with pathological compounds). Please don't send a pool of urines, except if urine has been collected on a short period of time from the same patient.

As soon as possible after collection, the urine sample must be heated at 50 °C for 20 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Then aliquot the sample in 10 ml plastic tubes (minimum 48 tubes), add stoppers and freeze. Be careful to constantly homogenize the urine while aliquoting the sample. Send the aliquots on dry ice by rapid mail or express transport to:

Mrs C Scott and Miss S Colyer
NHS
Department of Clinical
Chemistry and Newborn Screening
The Children's Hospital
Sheffield
S10 2TH
United Kingdom

Please send us an e-mail on the day you send the samples.

11. Tentative schedule and 2023

Sample distribution	8th February 2023
Start of analysis of Survey 2023/1 Website open	9th May 2023
Survey 2023/1 - Results submission	30th May 2023
Survey 2023/1 - Reports	June 2023
Start of analysis of Survey 2023/2	29th August 2023
Survey 2023/2 – Results submission	19th September 2023
Survey 2023/2 - Reports	October 2023
Annual Report 2023	January 2024

12. ERNDIM certificate of participation

A combined certificate of participation covering all EQA schemes will be provided to all participants who take part in any ERNDIM scheme.

Date of report, 2023-01-20
Name and signature of Scientific Advisor

Mrs C Scott and Miss S Colyer
NHS
Department of Clinical
Chemistry and Newborn Screening
The Children's Hospital
Sheffield
S10 2TH
United Kingdom

APPENDIX 1. **Change log (changes since the last version)**

Version Number	Published	Amendments
1	14 March 2023	2022 annual report published

END