ERNDIM Administration Office
c/o EMQN CIC, Unit 4, Enterprise House,
Manchester Science Park, Pencroft Way,
Manchester M15 6SE, United Kingdom.
Tel: +44 161 757 4952
Fax: +44 161 850 1145
Email: admin@erndim.org

Scientific Coordination
Petr Chrastina
Department of Pediatrics and Inherited Metabolic Disorders
General University Hospital in Prague
Ke Karlovu 2
128 08 Prague 2
Czech Republic
Tel: +420 224 947 161
Email: petr.chrastina@vfn.cz

Scheme Organisation
1) Alessandro Salemma; 2) Nicola Braik
CSCQ, Swiss Center for Quality Control
2 chemin du Petit-Bel-Air
CH-1225 Chêne-Bourg
Switzerland
Email: 1) Alessandro.Salemma@hcuge.ch
2) Nicola.Braik@hcuge.ch

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Diagnostic Proficiency Testing

Centre: Czech Republic

Final Report 2022

prepared by
Petr Chrastina

Note: This annual report is intended for participants of the ERNDIM DPT Czech Republic 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 terms and conditions in the ERNDIM Privacy Policy on www.erndim.org.

1. Geographical distribution of participants

Nineteen laboratories from 13 countries have participated in the Diagnostic Proficiency Testing scheme in 2022, for details see the below table:

Country	Number of participants
Austria	1
Croatia	1
Cyprus	1
Czechia	1
Denmark	1
Finland	1
Germany	6
Latvia	1
Lithuania	1
Malaysia	1
Netherlands	1
Portugal	1
Slovakia	2

[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.

2. Design and logistics of the scheme including sample information

The scheme has been designed and planned by Petr Chrastina as Scientific Advisor and coordinated by Alessandro Salemma as scheme organiser (sub-contractor on behalf of CSCQ), both appointed by and according to procedures laid down the ERNDIM Board.
CSCQ dispatches DPT EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports. Existing DPT and Urine MPS 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 six urines were obtained from patients with known diagnoses. Four urine samples have been provided by the scheme organizers and one sample has been provided by Department of Clinical Biochemistry of University Children's Hospital in Bratislava. The common sample was from DPT center UK (distributed in all five DPT schemes).

In 2022 the samples have been heat-treated and apart from the common sample A were re-analyzed in our department after receiving the samples from CSCQ (samples were shipped via courier after 3 days at ambient temperature to mimic possible changes that might arise during transport). In all six samples prepared and checked by us the typical metabolic profiles were preserved after heat treatment and shipment from CSCQ.
Mailing: samples were sent by DHL; FedEx or the Swiss Post at room temperature.

3. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines were required in 2022.

4. Schedule of the scheme

Sample distribution by CSCQ	02 February 2022
Start of analysis of Survey 2022/1	14 March 2022
Survey 2022/1 – results submission	04 April 2022
Survey 2022/1 – report	10 May 2022
Start of analysis of Survey 2022/2	06 June 2022
Survey 2022/2 – results submission	28 June 2022
Survey 2022/2 – report	02 August 2022
Annual meeting of participants	30 August 2022
Annual report 2022	December 2022

5. Results

18 of 19 labs returned results for both surveys by the deadline.

	Survey 1	**Survey 2**
Receipt of results	19	18
No answer	0	1

6. Web site reporting

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

- Selection of tests: **don't select a test if you will not perform it**, otherwise the evaluation program includes it in the report.
- Results
  - Give quantitative data as much as possible.
  - Enter the key metabolites with the evaluation **in the tables** even if you don't give quantitative data.

- 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.
  - **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 DPT Czech Republic 2022 have been also scored by Christine Saban, from DPT France. At the SAB meeting on 24th November 2022, the definitive scores have been finalized.

ERNDIM is now applying the concept of ‘critical error’ in the scoring of results. In principle this is a category of error that would be unacceptable to the majority of labs and would have a serious adverse effect on patient management.
Examples of such errors could be:
a) Failure to recognise a pre-defined set of diagnoses.
b) Missing a diagnosis when proficiency for that EQA sample is >95% (where proficiency for an EQA sample is the percentage of EQA participants that correctly identified the diagnosis of the sample).
c) Failure to perform a relevant test.
d) Identifying a ‘normal’ sample as having an IEM when it is clear that the sample was obtained from a patient not suspected of having an IEM and the findings reported were not identified by the rest of the participants and this diagnosis could potentially result in treatment that is harmful for the patient.

When a critical error is established for one or more samples, performance is not acceptable in that year, regardless of the number of points assigned. A critical error needs to be ratified by the ERNDIM Scientific Advisory Board. There was one critical error in 2022.

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. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

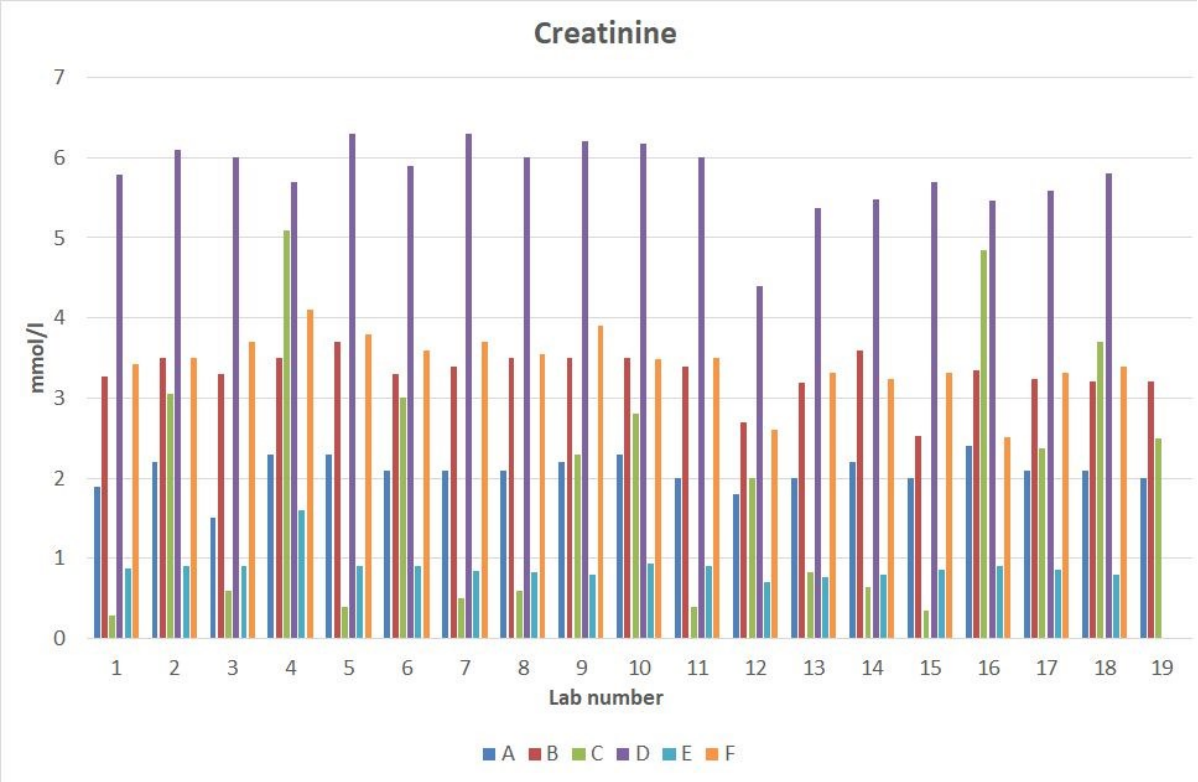
From 2022, performance of the participant that obtained at least 17 points from the maximum of 24 (71%) and more within the calendar year and that did not receive “critical error” mark is considered satisfactory.

If your laboratory is assigned poor performance and you wish to appeal against this classification, please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was mostly satisfying. In sample C from patient with alkaptonuria, many labs reported low creatinine concentration. It may have been because high concentration of homogentic acid interfere with creatinine measurement mainly with enzymatic method.



Sample	A	B	C	D	E	F
mean	2,08	3,31	1,91	5,79	0,89	3,44
median	2,10	3,35	2,00	5,85	0,87	3,49
SD	0,21	0,29	1,56	0,45	0,19	0,39

8.2. Patient A

Barth syndrome (3-methylglutaconic aciduria type II)

Patient details provided to participants

Pre-natal growth concerns. Monitored throughout life for poor growth. Presented at 4 years of age due to lips going blue during exercise.

Patient details

The sample was obtained from a 14-years old boy with Barth syndrome due to mutation in the tafazzin gene. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance

All participants analyzed organic acids and 18 of them reported elevated excretion of 3-methylglutaconic

acid. Such analytical finding was considered correct result and scored with 2 points. The analytical performance for this sample was very good (95%).

Interpretative proficiency and recommendation
The diagnosis of Barth syndrome was considered correct (2 points), while suspicion for other types of 3-methylglutaconic aciduria or non-specified 3 methylglutaconic aciduria was considered helpful but incomplete (1 point). Confirmation of diagnosis by mutation analysis of TAFAZZIN gene were considered helpful. The proficiency score for this sample was very good (92%).

Critical errors
The failure to recognize abnormal excretion of 3-methylglutaconic acid is considered by the ERNDIM SAB as a critical error, which would prevent establishing the correct diagnosis; critical error was assigned to one participant in our scheme.

Overall impression

Typical DPT sample with very good proficiency score (93%).

8.3. Patient B

Mucopolysaccharidosis type VII due to beta-glucuronidase deficiency

Patient details provided to participants
A 5 months old boy presented with hepatosplenomegaly and vertebrae deformities. The sample was collected at the age of 14 years.

Patient details
The sample was obtained from a 14-years old boy with mucopolysaccharidosis type VII due to beta-glucuronidase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance
18 participants analyzed glycosaminoglycans (GAG) in urine and 14 participants performed GAG fractionation. Elevated excretion of glycosaminoglycans without report on chondroitin sulfate and/or dermatan sulfate elevation was considered as partially correct and scored with 1 point. Increased proportion of chondroitin sulfate and/or dermatan sulphate was scored as correct analytical result with 2 points. The analytical performance for this sample was slightly suboptimal (61%).

Interpretative proficiency and recommendation
The diagnosis of mucopolysaccharidosis type VII was considered correct (2 points), while suspicion for MPS (other types of MPS or non-specified MPS) was considered helpful but incomplete (1 point). The diagnosis of mucopolysaccharidosis type VII based on clinical information was scored with 1 point. Confirmation of diagnosis by measurement of beta-glucuronidase in leukocytes/fibroblasts and/or mutation analysis of GUSB gene were considered helpful. Recommendation to carry out GAG fractionation for those participants that did not perform this analysis was considered also helpful (1 point). The proficiency score for this sample was slightly suboptimal (66%).

Critical errors
No critical error for this sample.

Overall impression
Typical DPT sample with slightly suboptimal proficiency score (63%).

8.4. Patient C

Alkaptonuria due to homogentisate 1,2-dioxygenase deficiency

Patient details provided to participants
This man was referred at the age of 53 years with arthritis. Dark brown urine and pigmentation in the cartilage of the ear and the sclera of the eye were noted. The sample was collected at the age of 53 years.

Patient details
This sample was obtained from a 53 years old man with alkaptonuria due to homogentisate 1,2-

dioxygenase deficiency, diagnosis was confirmed by molecular genetic analysis.

Analytical performance
All participants analyzed organic acids and all of them reported elevated excretion of homogentisic acid. Such analytical finding was considered correct result and scored with 2 points. The proficiency score for this sample was excellent (100%).

Interpretative proficiency and recommendation
Alkaptonuria was considered correct diagnosis and scored with 2 points. Confirmation of diagnosis by mutation analysis of HGD gene were considered helpful. The proficiency score for this sample was excellent (100%).

Critical errors
No critical error for this sample.

Overall impression
Easy DPT sample with excellent proficiency score (100%).

8.5. Patient D

Alpha-mannosidosis due to alpha-mannosidase deficiency

Patient details provided to participants
A 15 years old boy was referred for short stature, recurrent infections, scoliosis and hearing loss. The sample was collected at the age of 19 years.

Patient details
The sample was obtained from a 19-year-old man with alpha-mannosidosis due to alpha mannosidase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance
15 labs performed OLS analysis and 14 of them reported a correct analytical finding "OLS profile characteristic for alpha-mannosidosis", which was scored with 2 points. The analytical performance was slightly suboptimal (78%).

Interpretative proficiency and recommendation
The diagnosis of alpha-mannosidosis due to alfa-mannosidase deficiency was considered correct and scored with 2 points. Confirmation of diagnosis by enzyme assay of alfa-mannosidase activity in plasma/fibroblasts/leucocytes and/or mutation analysis of MAN2B1 gene were considered helpful. Recommendation to carry out oligosaccharide
analysis for those participants who did not perform this analysis was considered also helpful and scored with 1 point. The interpretative proficiency score for this sample was good (81%).

Critical errors
No critical error for this sample.

Overall impression
Typical DPT sample with slightly suboptimal proficiency score (79%).

8.6. Patient E

Argininosuccinic aciduria due to argininosuccinate lyase deficiency

Patient details provided to participants
A 13 years old boy was admitted to hospital for hepatopathy, lethargy, seizures, and hyperammonemia. The sample was collected at the age of 13 years on the specific treatment.

Patient details
The sample was obtained from a 13-year-old boy with argininosuccinic aciduria due to argininosuccinate lyase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance
All participants performed analysis of amino acids. 17 participants observed increased excretion of

argininosuccinic acid and/or its anhydrides, such analytical finding was considered correct and scored by 2 points. The analytical performance was very good (94%).

Interpretative proficiency and recommendation
The diagnosis of argininosuccinic aciduria due to argininosuccinate lyase deficiency was considered correct and scored with 2 points. Suspicion for urea cycle disorder was considered helpful but incomplete and scored with 1 point. Confirmation of diagnosis by enzyme assay of argininosuccinate lyase activity in erythrocytes /fibroblasts and/or mutation analysis of ASL gene were considered helpful. The interpretative proficiency score for this sample was very good (97%).

Critical errors
No critical error for this sample.

Overall impression
Easy DPT sample with very good proficiency score (96%).

8.7. Patient F

Aminoacylase 1 deficiency

Patient details provided to participants
A 9 years old boy was referred for psychomotor delay, hearing loss and cyclic vomiting. The sample was collected at the age of 11 years.

Patient details
This sample was obtained from an 11-year-old boy with aminoacylase 1 deficiency, diagnosis was confirmed by molecular genetic analysis.

Analytical performance
All participants analyzed organic acids and 12 of them reported elevated excretion of N-acetylated amino acids (N-acetylalanine, N-acetylmethionine, N-acetylglycine, N acetylglutamine, N-acetylglutamate, N-acetylserine, N-acetylvaline, N-acetylleucine, N acetylisoleucine, N-acetylthreonine) with normal excretion of N-acetylaspartate. Such analytical finding was considered correct result and scored with 2 points. The analytical performance for this sample was suboptimal (67%).

Interpretative proficiency and recommendation
The diagnosis of aminoacylase 1 deficiency was considered correct and scored with 2 points. Confirmation of diagnosis by enzymatic assay and/or mutation analysis of ACY1 gene were considered helpful. The proficiency score for this sample was suboptimal (67%).

Critical errors
No critical error for this sample.

Overall impression
Typical DPT sample with suboptimal proficiency score (67%).

Figure 1: organic acids profile (GC/MS) in urine of patient 2022F (heat-treated urine after 3 days at RT)

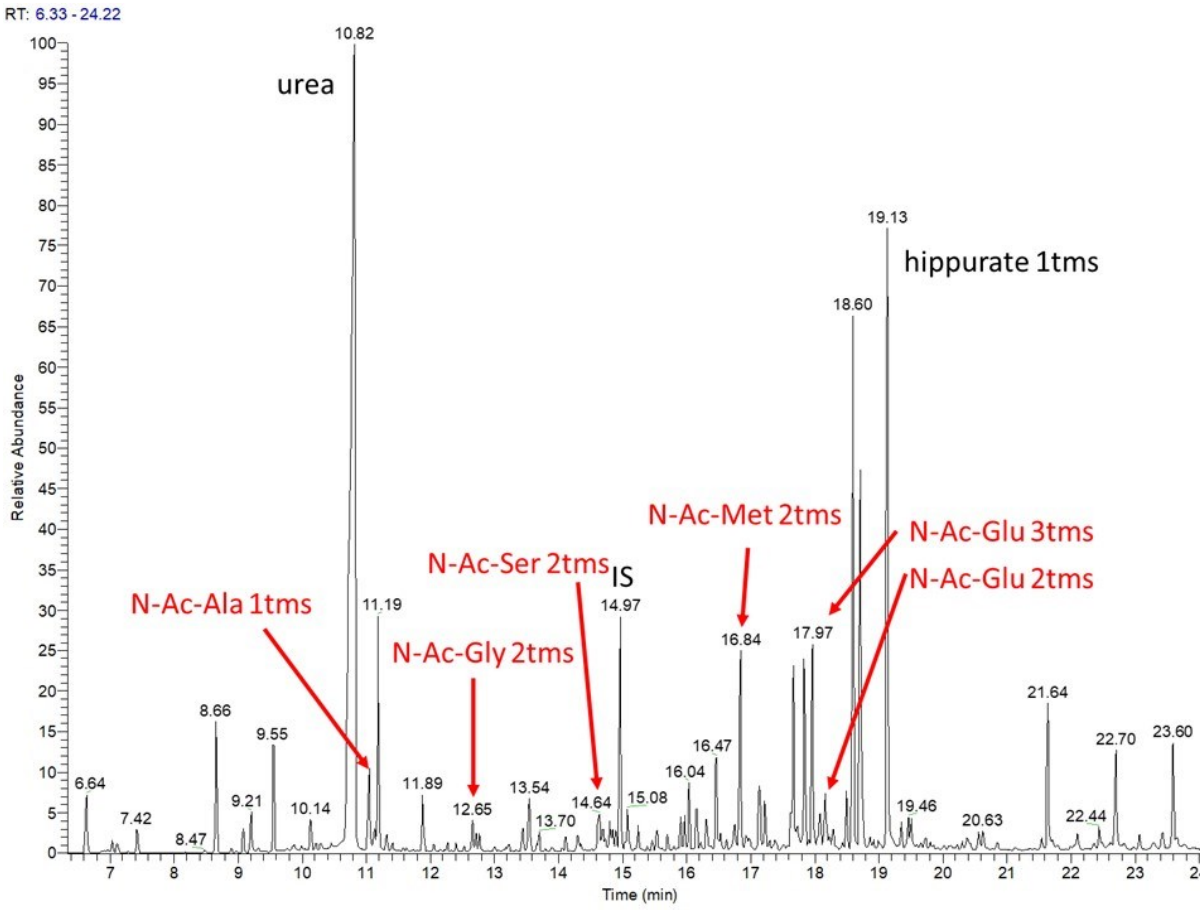
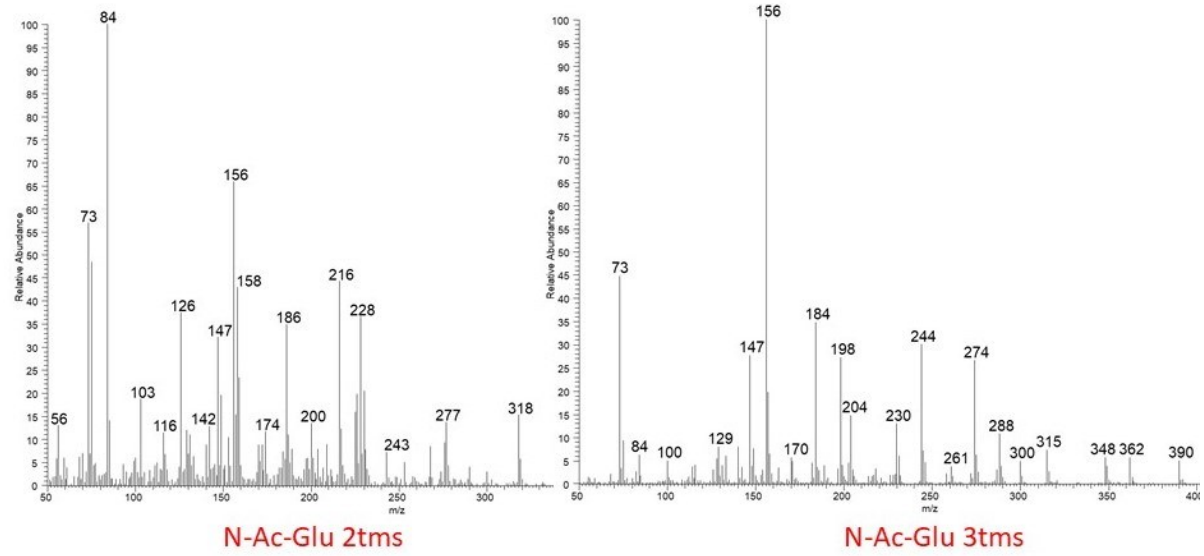
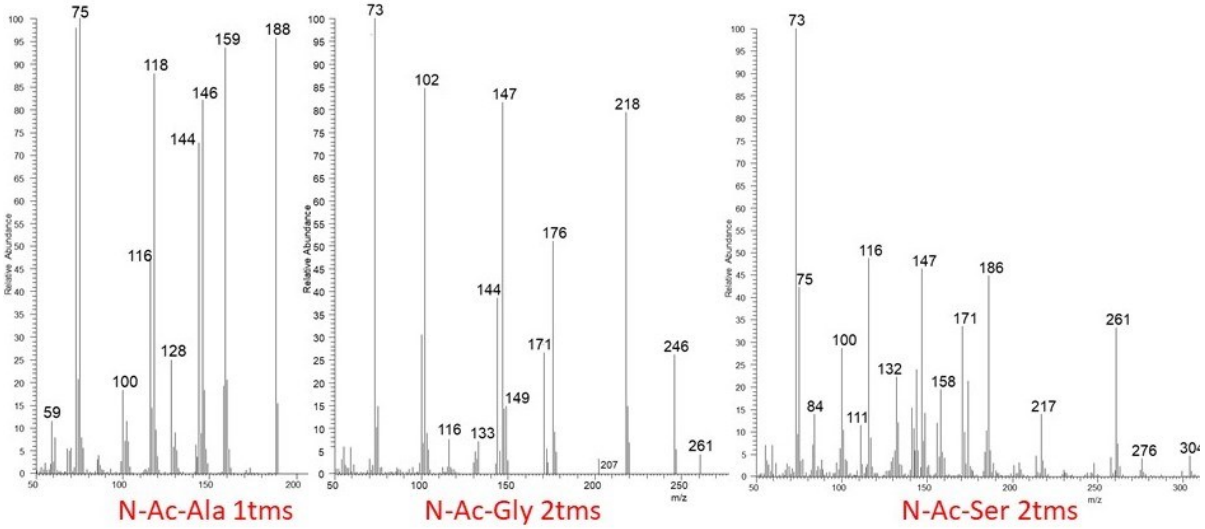
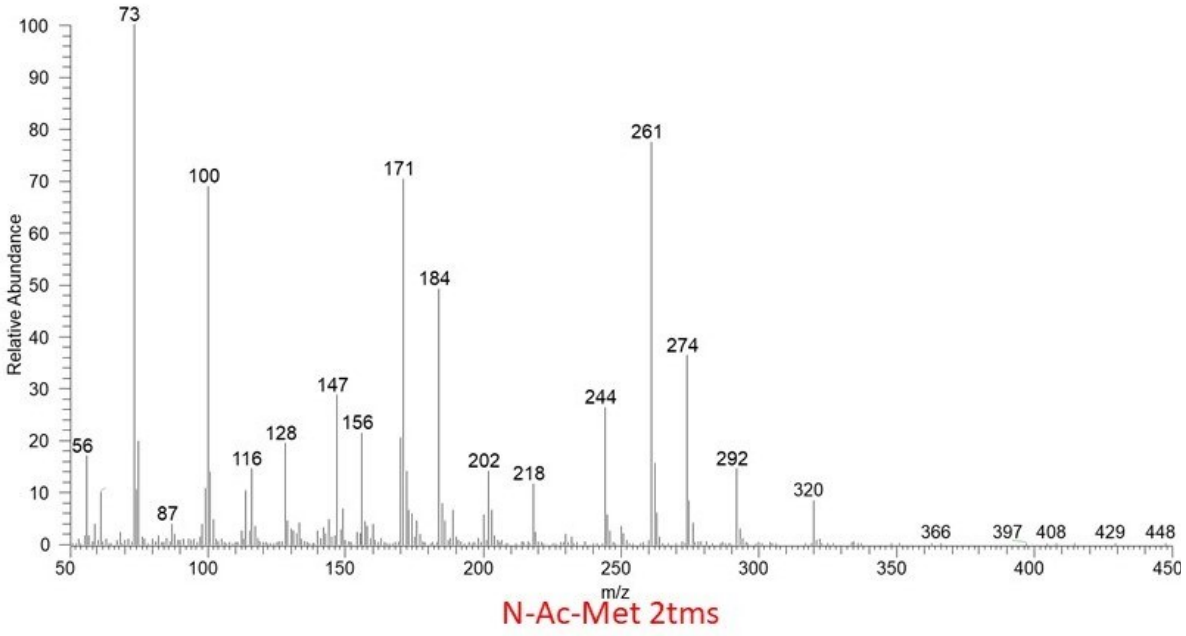


Figure 2: EI mass spectra of N-acetylated amino acids




N-Ac-Ala 1tms
N-Ac-Gly 2tms
N-Ac-Ser 2tms


N-Ac-Met 2tms
Relative Abundance
m/z

9. Scores of participants

All data transfer, the submission of data as well as the request and viewing of reports proceed via the DPT-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.

Detailed scores – Round 1

Lab n°	Patient A Barth syndrome			Patient B Mucopolysaccharidosis type VII			Patient C Alkaptonuria			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	2	2	4	12
2	2	2	4	2	2	4	2	2	4	12
3	2	2	4	0	0	0	2	2	4	8
4	2	2	4	1	1	2	2	2	4	10
5	2	2	4	2	2	4	2	2	4	12
6	2	2	4	1	2	3	2	2	4	11
7	2	2	4	1	1	2	2	2	4	10
8	2	2	4	1	1	2	2	2	4	10
9	2	2	4	2	2	4	2	2	4	12
10	0	0	0	1	1	2	2	2	4	6
11	2	2	4	0	1	1	2	2	4	9
12	2	2	4	1	1	2	2	2	4	10
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	2	2	4	12
15	2	2	4	2	1	3	2	2	4	11
16	2	1	3	1	1	2	2	2	4	9
17	2	2	4	0	1	1	2	2	4	9
18	2	2	4	1	1	2	2	2	4	10
19	2	2	4	1	1	2	2	2	4	10

Detailed scores – Round 2

Lab n°	Patient D Alpha-mannosidosis			Patient E Argininosuccinic aciduria			Patient F Aminoacylase 1 deficiency			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	2	2	4	12
2	2	2	4	2	2	4	2	2	4	12
3	2	2	4	2	2	4	0	0	0	8
4	2	2	4	2	2	4	2	2	4	12
5	0	0	0	0	1	1	2	2	4	5
6	2	2	4	2	2	4	2	2	4	12
7	2	2	4	2	2	4	2	2	4	12
8	2	2	4	2	2	4	0	0	0	8
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	2	2	4	0	0	0	8
11	0	1	1	2	2	4	2	2	4	9
12	0	0	0	2	2	4	2	2	4	8
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	0	0	0	8
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	2	2	4	2	2	4	12
17	2	2	4	2	2	4	0	0	0	8
18	0	0	0	2	2	4	0	0	0	4
19	--	--	--	--	--	--	--	--	--	0

Total scores

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)	Critical error
1	4	4	4	4	4	4	24	100	
2	4	4	4	4	4	4	24	100	
3	4	0	4	4	4	0	16	67	
4	4	2	4	4	4	4	22	92	
5	4	4	4	0	1	4	17	71	
6	4	3	4	4	4	4	23	96	
7	4	2	4	4	4	4	22	92	
8	4	2	4	4	4	0	18	75	
9	4	4	4	4	4	4	24	100	
10	0	2	4	4	4	0	14	58	CE
11	4	1	4	1	4	4	18	75	
12	4	2	4	0	4	4	18	75	
13	4	4	4	4	4	4	24	100	
14	4	4	4	4	4	0	20	83	
15	4	3	4	4	4	4	23	96	
16	3	2	4	4	4	4	21	88	
17	4	1	4	4	4	0	17	71	
18	4	2	4	0	4	0	14	58	
19	4	2	4	--	--	--	10	42	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	15	79
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	3	16
Partial and non-submitters	1	5

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-CP-2022-A	Barth syndrome	95	92	93
DPT-CP-2022-B	Mucopolysaccharidosis type VII	61	66	63
DPT-CP-2022-C	Alkaptonuria	100	100	100
DPT-CP-2022-D	Alpha-mannosidosis	78	81	79
DPT-CP-2022-E	Argininosuccinic aciduria	94	97	96
DPT-CP-2022-F	Aminoacylase 1 deficiency	67	67	67

10. Annual meeting of participants

The annual meeting of participants of the Proficiency Testing Centre Czech Republic was held during SSIEM Annual Symposium on 30th August 2022 in Freiburg, Germany.

This year we encountered one major analytical difficulty, absent annotation of elevated excretion of N-acetylated amino acids in sample F.

We remind you that attending the annual meeting is an important part of the proficiency testing. The goal of the program is to **improve** the competence of the participating laboratories, which includes the critical review of all results with a discussion about improvements.

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

- **Training**: SSIEM ACADEMY
  - The 2023 SSIEM Academy Clinical and Laboratory Scientist courses will be held in Manchester, United Kingdom, on the 24th and 25th April 2023. The program includes:
    - Organic acidemias
    - Fatty acid oxidation defects
    - Metabolic Cardiomyopathies
  - The lectures will be available on the SSIEM website
- **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. If possible, please collect 1500 ml of urine: this sample can be sent to all labs participating to one of the DPT schemes. 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. For "normal" urine, the sample must be collected from a symptomatic patient (don't send urine from your kids!). As soon as possible after collection, the urine sample must be heated at 56 °C for 30 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Please send us an e-mail about possible samples and we will organize transport with your cooperation.

12. Reminders

We remind you that to participate to the DPT-scheme, you must perform at least:

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides

If you are not performing one of these assays, you can send the samples to another lab (cluster lab) but you are responsible for the results.
Please send quantitative data for amino acids and, as much as possible, for organic acids.

13. Tentative schedule and fee in 2023

Sample distribution	07 February 2023
Start of analysis of Survey 2023/1	13 March 2023
Survey 2023/1 – results submission	03 April 2023
Survey 2023/1 – report	15 May 2023
Start of analysis of Survey 2023/2	05 June 2023
Survey 2023/2 – results submission	26 June 2023
Survey 2023/2 – report	07 August 2023
Annual meeting of participants	29 August 2023
Annual report 2023	January 2024

14. 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. For the DPT scheme this certificate will indicate if results were submitted and whether satisfactory performance was achieved in the scheme.

Date of report, 2022-12-29
Name and signature of Scientific Advisor

Petr Chrastina
Department of Pediatrics and Inherited Metabolic Disorders
General University Hospital in Prague
Ke Karlovu 2
128 08 Prague 2
Czech Republic
Tel: +420 224 947 161
Email: petr.chrastina@vfn.cz

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

Version Number	Published	Amendments
1	16 January 2023	2022 annual report published

END