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Diagnostic Proficiency Testing

Centre: Czech Republic

Final Report 2023

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 2023, for details see the below table:

Country	Number of participants
Austria	1
Croatia	1
Cyprus	1
Czech Republic	1
Denmark	1
Finland	1
France	1
Germany	6
Latvia	1
Lithuania	1
Malaysia	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. Three urine samples have been provided by the scheme organizers, one sample has been provided by Clinical Institute of Laboratory Diagnostics of Zagreb University Hospital Centre, one sample has been provided by Department of Endocrinology and Metabolism of Labor Berlin and one sample has been provided by Department of Clinical Biochemistry of University Children's Hospital in Bratislava. The common sample was from our DPT center (distributed in all five DPT schemes).

In 2023 all six samples have been heat-treated 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 2023.

4. Schedule of the scheme

Sample distribution by CSCQ	08 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

5. Results

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

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

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 2023 have been also scored by Déborah Mathis, from DPT Switzerland. At the SAB meeting on 30th November 2023, 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 2023.

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

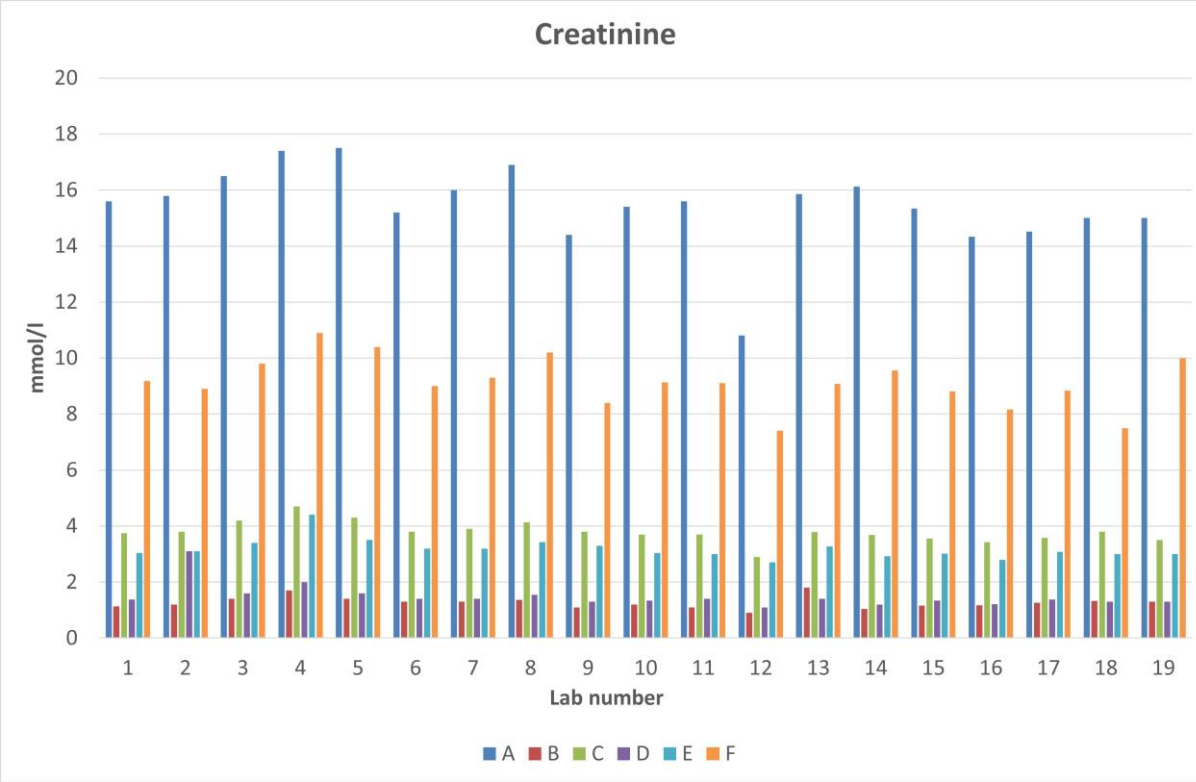
From 2023, performance of the participant that obtained at least 17 points from the maximum of 24 (71%) and more within the calendar year and that did not receive “critical error” mark is considered satisfactory.

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 with some outliers.



Sample	A	B	C	D	E	F
mean	15.44	1.27	3.79	1.49	3.18	9.14
median	15.60	1.26	3.78	1.38	3.08	9.10
SD	1.45	0.21	0.38	0.43	0.36	0.90

8.2. Patient A

argininosuccinic aciduria due to argininosuccinate lyase deficiency

Patient details provided to participants

This boy was referred at the age of 7 years with attention deficit disorder. The sample was collected at the age of 25 years on the specific treatment.

Patient details

The sample was obtained from a 25-years old man with mild form of argininosuccinic aciduria due to argininosuccinate lyase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance

18 participants analyzed amino acids and 14 of them reported elevated excretion of argininosuccinic acid and/or its anhydrides. Such analytical finding was considered correct result and scored with 2 points. The analytical performance for this sample was suboptimal (74%).

Interpretative proficiency and recommendation

The diagnosis of argininosuccinic aciduria was considered appropriate and scored with 2 points. Although further confirmation of argininosuccinic aciduria is not necessary a confirmation of diagnosis by enzymatic assay and/or mutation analysis of ASL gene may be useful in case of prenatal diagnosis in the affected family. Recommendation to carry out amino acids analysis for those participants that did not perform this analysis was considered also helpful (1 point). The proficiency score for this sample was suboptimal (76%).

Critical errors

No critical error for this sample.

Overall impression

Typical DPT sample with suboptimal proficiency score (75%).

Figure 1: amino acids profile (IEC-NHD) in urine of patient 2023A (heat-treated urine after 3 days at RT)

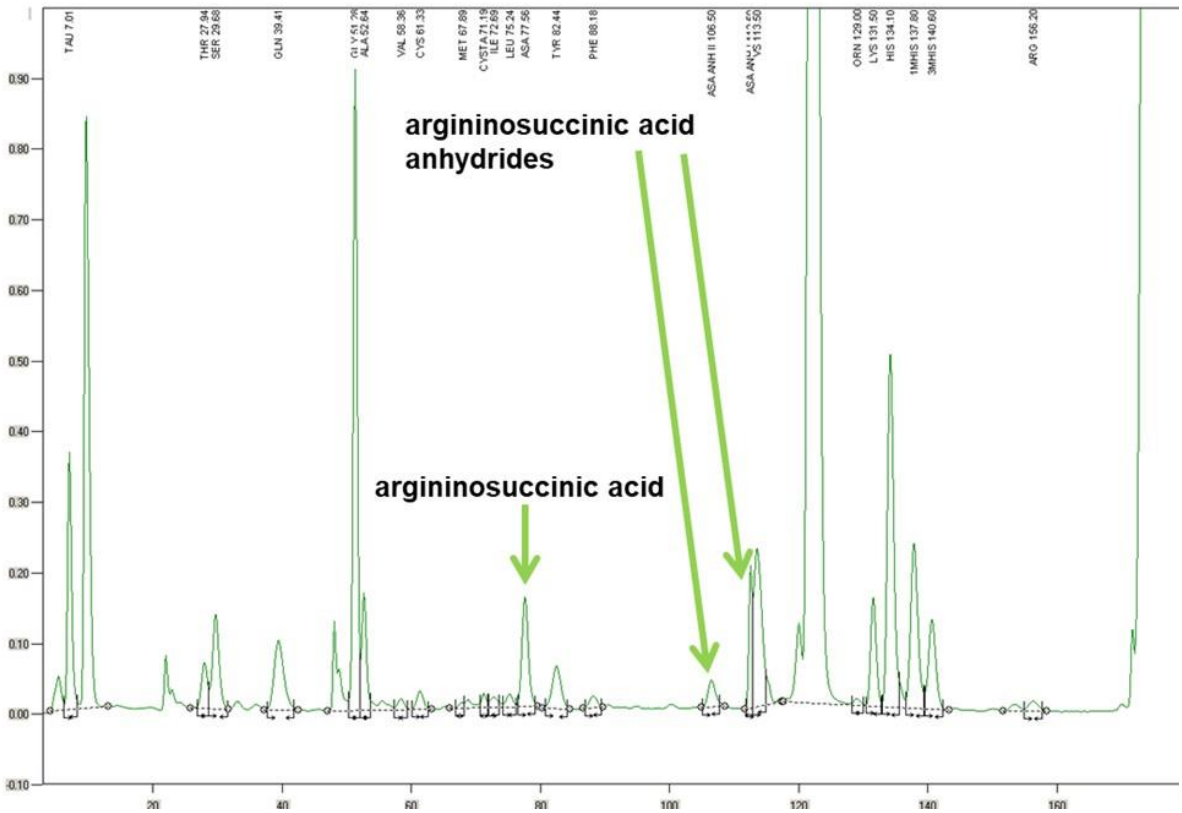
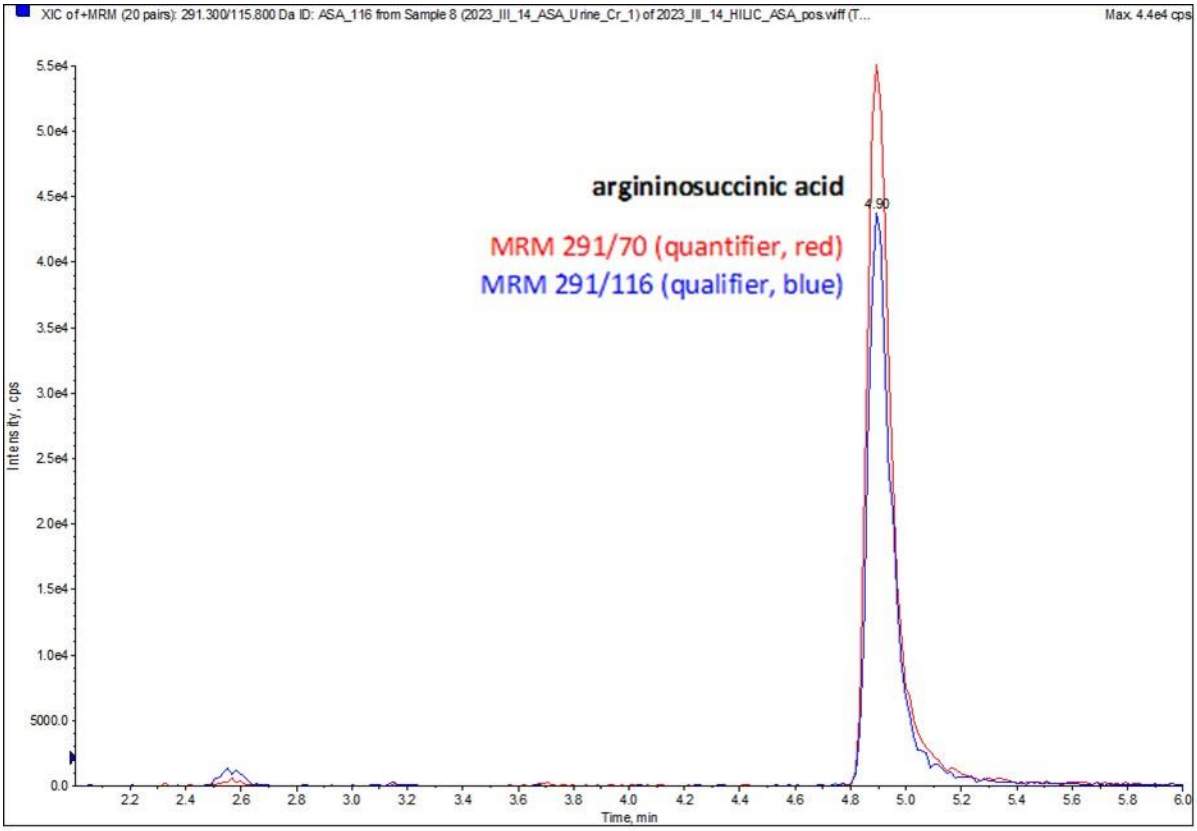


Figure 2: detection of argininosuccinic acid (LC-MS/MS) in urine of patient 2023A (heat-treated urine after 3 days at RT)



8.3. Patient B

3-hydroxy-3-methylglutaric aciduria due to 3-hydroxy-3-methylglutaryl-CoA lyase deficiency

Patient details provided to participants

A 5-year-old boy was admitted to hospital in coma with seizure and profound hypoglycemia. The sample was collected at the age of 6 years while he received specific treatment.

Patient details

The sample was obtained from a 6-years old boy with 3-hydroxy-3-methylglutaric aciduria due to 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance

All participants analyzed organic acids and 18 of them observed increased excretion of 3-hydroxy-3-methylglutaric acid, such analytical finding was considered correct and scored with 1 point. All participants also reported elevated excretion of other related metabolites, e.g. 3-methylglutaconic acid, 3-methylglutaric acid or 3-hydroxyisovaleric acid, such analytical finding was also considered correct and scored with 1 point. The analytical performance for this sample was very good (97%).

Interpretative proficiency and recommendation

The diagnosis of 3-hydroxy-3-methylglutaric aciduria was considered appropriate and scored with 2 points. Confirmation of diagnosis by enzyme assay of 3-hydroxy-3-methylglutaryl-CoA lyase activity in fibroblasts or lymphocytes and/or mutation analysis of HMGCL gene was 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 very good proficiency score (99%).

8.4. Patient C

dihydropyrimidine dehydrogenase deficiency

Patient details provided to participants

An 18-year-old woman was referred with suspicion for multiple sclerosis. Urine was collected at the age of 25 years.

Patient details

The sample was obtained from a 25-years old woman with dihydropyrimidine dehydrogenase deficiency. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance

18 labs analyzed organic acids and 14 labs performed analysis of purines and pyrimidines. All participants reported elevated excretion of thymine (1 point) and uracil (1 point). The analytical performance was excellent (100%).

Interpretative proficiency and recommendation

Dihydropyrimidine dehydrogenase deficiency was considered the correct diagnosis. Confirmation of diagnosis by enzyme assay of dihydropyrimidine dehydrogenase activity in fibroblasts or lymphocytes and/or mutation analysis of the *DPYD* gene was considered helpful. The interpretative 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

D-2-hydroxyglutaric aciduria

Patient details provided to participants

This boy was referred at the age of 9 years because of macrocephaly and mental retardation. The sample was collected at the age of 9 years.

Patient details

The sample was obtained from from a 9-years old male patient with D-2-hydroxyglutaric aciduria. Absolute configuration of 2-hydroxyglutaric acid was established by 13C high-resolution NMR spectra.

Analytical performance

All participants analyzed organic acids and 18 of them observed increased excretion of 2-hydroxyglutaric acid, such analytical finding was considered correct and scored with 2 points. The analytical performance for this sample was very good (95%).

Interpretative proficiency and recommendation

The diagnosis of D-2-hydroxyglutaric aciduria or 2-hydroxyglutaric aciduria were considered appropriate and scored with 2 points. Suspicion for L-2-hydroxyglutaric aciduria only was considered helpful but incomplete and scored with 1 point. Confirmation of diagnosis by mutation analysis of D2HGDH and IDH2 genes was considered helpful. The proficiency score for this sample was good (87%).

Critical errors

No critical error for this sample.

Overall impression

Easy DPT sample with very good proficiency score (91%).

8.6. Patient E

phenylketonuria due to phenylalanine hydroxylase deficiency

Patient details provided to participants

A 47-year-old man was referred with severe intellectual disability, behavioral disturbances, and difficulty walking. The sample was collected at the age of 48 years on treatment.

Patient details

The sample was obtained from an untreated 48-year-old man with phenylketonuria due to phenylalanine hydroxylase deficiency, diagnosis was confirmed by molecular genetic analysis.

Analytical performance

18 participants analyzed urinary amino acids and reported high excretion of phenylalanine, such analytical finding was considered correct and scored by 1 point. All participants performed analysis of organic acids and 18 of them observed the high excretion of metabolites typical for PKU (phenyllactate, phenylpyruvate, 2-hydroxy-phenylacetate etc.), such analytical finding was also considered correct and scored by 1 point. The analytical performance for this sample was very good (95%).

Interpretative proficiency and recommendation

Phenylketonuria was considered the correct diagnosis and scored with 2 points. Confirmation of diagnosis by mutation analysis of PAH gene was considered helpful. The proficiency score for this sample was very good (95%).

Critical errors

The failure to recognize abnormal excretion of phenylalanine and/or its metabolites 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

Easy DPT sample with very good proficiency score (95%).

8.7. Patient F

cystinuria

Patient details provided to participants

A 15-year-old boy was referred with kidney stones. The sample was collected at the age of 16 years while he received specific treatment.

Patient details

The sample was obtained from 16-years old male with cystinuria. The diagnosis was confirmed by molecular genetic analysis.

Analytical performance

All participants analyzed urinary amino acids. All participants reported presence of increased excretion of cystine, such analytical finding was considered a correct analytical result and scored by 1 point. 18 participants reported dibasic hyperaminoaciduria, such analytical finding was considered a correct analytical result and scored by 1 point. The analytical performance was very good (97%).

Interpretative proficiency and recommendation

Cystinuria was considered the correct diagnosis and scored with 2 points. Confirmation of diagnosis by mutation analysis of SLC3A1 and SLC7A9 genes was 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 very good proficiency score (99%).

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.

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. Details of how to appeal poor performance are included in the Performance Support Letter sent to poor performing laboratories

Detailed scores – **Round 1**

Lab n°	Patient A argininosuccinic aciduria			Patient B 3-hydroxy-3-methylglutaric aciduria			Patient C dihydropyrimidine dehydrogenase 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	1	2	3	2	2	4	11
3	2	2	4	2	2	4	2	2	4	12
4	2	2	4	2	2	4	2	2	4	12
5	2	2	4	2	2	4	2	2	4	12
6	2	2	4	2	2	4	2	2	4	12
7	2	2	4	2	2	4	2	2	4	12
8	0	0	0	2	2	4	2	2	4	8
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	2	2	4	2	2	4	12
11	2	2	4	2	2	4	2	2	4	12
12	2	2	4	2	2	4	2	2	4	12
13	2	2	4	2	2	4	2	2	4	12
14	0	0	0	2	2	4	2	2	4	8
15	0	1	1	2	2	4	2	2	4	9
16	2	2	4	2	2	4	2	2	4	12
17	2	2	4	2	2	4	2	2	4	12
18	0	0	0	2	2	4	2	2	4	8
19	0	0	0	2	2	4	2	2	4	8

Detailed scores – Round 2

Lab n°	Patient D D-2-hydroxyglutaric aciduria			Patient E phenylketonuria			Patient F cystinuria			
	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	2	2	4	12
4	2	1	3	2	2	4	2	2	4	11
5	2	0	2	2	2	4	2	2	4	10
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	2	2	4	12
9	2	2	4	2	2	4	2	2	4	12
10	0	0	0	2	2	4	2	2	4	8
11	2	2	4	2	2	4	2	2	4	12
12	2	2	4	2	2	4	2	2	4	12
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	2	4	2	2	4	12
16	2	2	4	2	2	4	2	2	4	12
17	2	2	4	2	2	4	1	2	3	11
18	2	2	4	2	2	4	2	2	4	12
19	2	2	4	0	0	0	2	2	4	8

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	3	4	4	4	4	23	96	
3	4	4	4	4	4	4	24	100	
4	4	4	4	3	4	4	23	96	
5	4	4	4	2	4	4	22	92	
6	4	4	4	4	4	4	24	100	
7	4	4	4	4	4	4	24	100	
8	0	4	4	4	4	4	20	83	
9	4	4	4	4	4	4	24	100	
10	4	4	4	0	4	4	20	83	
11	4	4	4	4	4	4	24	100	
12	4	4	4	4	4	4	24	100	
13	4	4	4	4	4	4	24	100	
14	0	4	4	4	4	4	20	83	
15	1	4	4	4	4	4	21	88	
16	4	4	4	4	4	4	24	100	
17	4	4	4	4	4	3	23	96	
18	0	4	4	4	4	4	20	83	
19	0	4	4	4	0	4	16	67	CE

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 70 % of adequate responses)	18	95
Unsatisfactory performers (< 70 % adequate responses and/or critical error)	1	5
Partial and non-submitters	0	0

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-CP-2023-A	argininosuccinic aciduria	74	76	75
DPT-CP-2023-B	3-hydroxy-3-methylglutaric aciduria	97	100	99
DPT-CP-2023-C	dihydropyrimidine dehydrogenase deficiency	100	100	100
DPT-CP-2023-D	D-2-hydroxyglutaric aciduria	95	87	91
DPT-CP-2023-E	phenylketonuria	95	95	95
DPT-CP-2023-F	cystinuria	97	100	99

10. Annual meeting of participants

The annual meeting of participants of the Proficiency Testing Centre Czech Republic was held during SSIEM Annual Symposium on 29th August 2023 in Jerusalem, Israel.

This year we encountered one major analytical difficulty, absent annotation of elevated excretion of argininosuccinic acid and its anhydrides in sample A.

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

- **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 2018

Sample distribution	07 February 2024
Start of analysis of Survey 2024/1	12 March 2024
Survey 2024/1 – results submission	02 April 2024
Survey 2024/1 – report	14 May 2024
Start of analysis of Survey 2024/2	03 June 2024
Survey 2024/2 – results submission	24 June 2024
Survey 2024/2 – report	05 August 2024
Annual meeting of participants	27 August 2024
Annual report 2024	January 2025

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.

15. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the Scientific Advisor of the scheme, Petr Chrastina (petr.chrastina@vfn.cz) and/or to the ERNDIM Administration Office (admin@erndim.org)

Date of report, 2024-01-16
Name and signature of Scientific Advisor

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

Version Number	Published	Amendments
1	17 January 2024	2023 annual report published

END