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Diagnostic Proficiency Testing

Centre: The Netherlands

Final Report 2023

prepared by
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The ERNDIM Diagnostic Proficiency Testing (DPT) Scheme is the ultimate external quality assessment scheme for biochemical genetics laboratories. In 2023, 18 labs participated in the Proficiency Testing Scheme NL.

1. Geographical distribution of participants

For both surveys, all 18 participants have submitted results.

Country	Number of participants
Australia	2
Belgium	5
Germany	1
Netherlands	6
New Zealand	1
South Africa	1
Switzerland	1
United Kingdom	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.

2. Design and logistics of the scheme including sample information

The scheme has been designed and planned by dr George Ruijter 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 by 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. 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 samples: Samples used in 2023 have been provided by:

- UZ Brussel, Brussels
- AUMC, Amsterdam
- dr Jasinge, Colombo, Sri Lanka
- dr Crastina, scientific advisor of DPT-CZ
- Erasmus MC, Rotterdam

Patient A: ASL deficiency
Patient B: ADSL deficiency
Patient C: MADD
Patient D: MPS IIIC
Patient E: No IMD
Patient F: MCEE + SR

Sample pre-treatment (heat-treatment) was performed in the Scientific Advisor's laboratory, while aliquoting and dispatch of the samples was done by the Scheme organiser. Before dispatch to participants one set of samples was sent to the Scientific Advisor and checked for quality. In all six samples the typical metabolic profiles were preserved.
Shipping: samples were sent by DHL, FedEx or the Swiss Post at room temperature.
The time allotted for submitting reports was 3 weeks after opening of the website. Clinical information on the samples was provided through the website.

3. Tests

The minimal required test panel for participation in any DPT scheme includes creatinine, dip stick, amino acids, organic acids, oligosaccharides, quantitative GAG screening and purines-pyrimidines. It is strongly recommended to have the following tests available for DPT-NL: GAG subtype analysis (by electrophoresis, TLC or LC-MS/MS), sialic acid, creatine-guanidinoacetate and polyols-sugars. Please note that in DPT schemes it is allowed to obtain results from partner laboratories when this is routine clinical practice. It is required to indicate in the report that results were obtained from a cluster lab.

4. Schedule of the scheme

- February 8, 2023: shipment of samples
- March 13, 2023: start analysis of samples of the first survey
- April 3, 2023: deadline for result submission (Survey 1)
- May 15, 2023: interim report with preliminary scores of Survey 1 published
- June 5, 2023: start analysis of samples of the second survey
- June 26, 2023: deadline for result submission (Survey 2)
- August 7, 2023: interim report with preliminary scores of Survey 2 published
- September 19, 2023: online DPT meeting
- January 3, 2024: annual report with final scoring published

5. Results

All participants submitted results for both surveys on time.

	Survey 1	Survey 2
Receipt of results	18	18
No results submitted	0	0

6. Web site reporting

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

- Selection of tests: please **don't select a test if you do not intend to perform it**, otherwise the evaluation program will include it in the report.
- Results: please
  - Give quantitative data as much as possible.
  - Enter the key metabolites with interpretation **in the tables** even if you don't provide 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 investigations**)
  - Recommendations are scored together with interpretation.
  - Advice for treatment is not scored.
  - **Please don't give advice for further investigations in "Comments on diagnosis"**: it will not be included in the evaluation software.

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 aspects 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 the sum of these two aspects. The maximum score is 4 points per sample. The scores were calculated only for laboratories submitting results for both surveys.

Scoring and certificate of participation
Scoring is carried out by the scientific advisor and a second assessor from another DPT scheme. The second assessor changes every year. The results of DPT NL 2023 were additionally scored by Dr Petr Crastina, from DPT CZ. At the SAB meeting in Prague, November 30 – December 1, 2023, the definitive scores have been set. 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. Details on critical errors in the 2023 samples are given in section 8 of this report.

ERNDIM provides a single certificate for all its schemes with details of participation and performance. In addition, performance support letters will be issued if the performance is evaluated as unsatisfactory. One performance support letter will be sent by the Scheme Advisor for 2023. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

For DPT 2023 a total score of at least 14 points out of the maximum of 20 (70%) and absence of critical errors must be achieved for satisfactory performance. The SAB has decided during its meeting in Prague, November 30 – December 1, 2023, that sample 2023-F will be educational, i.e. not included in performance assessment.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was mostly correct for all labs. One lab had a systematic error in survey one with all three values being too high (treated as outliers). Creatinine values are provided in the Table below. CVs are <6 % for samples B, C, D and F, but slightly higher (8%) in samples A and E.

Sample	Median creatinine (mmol/L)	SD (mmol/L)	CV (%)	n
A	15.7	1.3	8.0	17
B	7.0	0.4	5.5	17
C	7.5	0.4	5.7	17
D	4.9	0.2	4.7	18
E	2.8	0.2	7.9	18
F	2.7	0.1	4.4	18

8.2. Patient A – Argininosuccinic acduria due to ASL deficiency (OMIM 207900)

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
This young man was diagnosed with a mild form of argininosuccinic aciduria due to argininosuccinate lyase deficiency. The diagnosis was confirmed by molecular genetic analysis. At the time the urine sample was collected he was treated with low protein diet (0,6 g/kg body weight) and did not have a metabolic decompensation. He was obese and suffers from hypertension and depression.

Sample A was the common sample distributed to participants of all 5 DPT centres and was discussed during the ERNDIM participant meeting in Jerusalem, August 29, 2023 by dr Petr Chrastina from Prague and summarised during the online DPT meeting of DPT-NL. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Analytical performance
Elevated argininosuccinic acid (ASA) was reported by 15 labs (2 points), while only 3 also mentioned the presence of ASA anhydrides. The median value for ASA values was 61 mmol/mol (range 21-96). Presumably the large range of reported values is due to different methods being used (LC-MS, IEC) and variable anhydride formation in the IEC procedure. Orotic acid was reported normal by most participants (11/18) and borderline by one lab. Analytical performance was 83%.

Diagnosis / Interpretative proficiency
All labs that found the ASA, reported the correct diagnosis (ASL deficiency; 2 points). The relatively low ASA level might explain three participants missing the diagnosis. Interpretation proficiency was 83%.

Other diagnoses reported were MSUD, tyrosinaemia type 3 and 'normal metabolic profile'.

Recommendations
Advice for further investigations included: blood ammonia, plasma amino acid analysis, enzyme assay in RBC/fibroblasts and mutation analysis of the ASL gene.

Scoring
- Analytical results: elevated argininosuccinic acid and/or anhydrides: score 2
- Interpretation of results: argininosuccinate lyase deficiency: score 2
- Critical error: sample not eligible

Overall impression
Overall proficiency in DPT-NL was 83%. ASA was not very high in the (concentrated) urine sample and across all DPT centres only 78 out of the 98 participants reported the correct diagnosis (80%), which is suboptimal for an ASL sample.

Multiple distributions of similar samples
A different ASA sample has been circulated in the DPT-NL scheme in 2011 and 2015. Proficiency was 95% in these years. As the argininosuccinate level was higher in the 2011/2015 samples, it was easier to establish diagnosis.

8.3. Patient B – Adenylosuccinate lyase deficiency (OMIM 103050)

Patient details provided to participants
Male, aged 20 y. Severe intellectual disability, convulsions and autism.

Patient details
This patient was diagnosed with ADSL deficiency at age 20 y by metabolic screening, which was confirmed by enzyme analysis in cultured fibroblasts. Recently genetic analysis showed two ADSL mutations. He was 47 y old at the time the DPT urine was sampled and living in sheltered housing.

Analytical performance
Fifteen participants reported elevated succinyl-aminoimidazolecarboxamide riboside (SAICAr) and/or succinyladenosine (S-Ado), which was scored with two points. Analytical proficiency was 83%.
Relatively small amounts of S-Ado and SAICAr were present in sample B. The median SAICAr level was 14 mmol/mol creat (range 9-16), S-Ado was 27 mmol/mol creat (range 21-33). A SAICAr calibrator is now commercially available. It is too expensive to include in the ERNDIM quantitative purine-pyrimidine (PPU) scheme, but its analogue AICAr is included in the PPU scheme. S-Ado can be prepared easily from adenylosuccinate (succinyl-AMP, available from Sigma). Reference values of S-Ado are age-dependent. Urine from young children usually does contain some S-Ado, which makes quantitative analysis imperative to identify ADSL patients.
The Bratton-Marshall test to detect SAICAr was reported by two participants, with one positive and one negative result. Perhaps the SAICAr level (approx. 100 µmol/l in this sample) was too low to obtain a robust positive Bratton-Marshall test, although the test has been reported to have an LOD of 1 µmol/l. These results indicate that this screening test is not reliable and we recommend to perform quantitative purine analysis.
Twelve participants reported elevated ethylmalonic acid (median value 16 mmol/mol creat). This is not related to the diagnosis and this finding has not been investigated further.

Diagnosis / Interpretative proficiency
The number of participants that came to the correct diagnosis for this sample was 15, which is explained by the fact that three participants did not perform purine analysis, while this is required for participation in DPT. Interpretative proficiency was 83%.
Three forms of ADSL deficiency are generally distinguished, i.e. a neonatal lethal form, a clinically severe form in which affected patients excrete roughly the same amounts of SAICAr and S-Ado and an attenuated form in which the urine S-Ado level is usually at least 2-fold higher than that of SAICAr. Apparently the present patient has the attenuated form of the disease.
Two participants reported SCAD deficiency as the most likely diagnosis, based on the slightly elevated ethylmalonic acid, and one participant reported ‘no diagnosis’. Evidence is accumulating in the scientific literature that SCAD deficiency is not disease-causing (e.g. Breilyn et al, Mol Gen Metab 138 (2023) 106971).

Recommendations
Advice for further investigations included measuring ADSL activity in red blood cells, leukocytes or fibroblasts and mutation testing of the ADSL gene. Follow-up of the slightly elevated ethylmalonic acid was also suggested (repeat OA, plasma acylcarnitines, thiosulfate, mutation testing), but the utility of this is questionable.

Scoring
- Analytical results: elevated SAICAr and/or S-Ado: score 2
- Interpretation of results: ADSL deficiency: score 2
- Critical error: sample not eligible

Overall impression
Despite SAICAr and S-Ado concentrations being not very high in this urine sample, most participants correctly established the diagnosis. Clearly, quantitative PuPy analysis is required to reach diagnosis. Overall proficiency (based on scores) was 83%.

Multiple distributions of similar samples
A different ADSL sample was circulated in 2012 (2012-D) with overall proficiency 62%.

8.4. Patient C – Multiple acyl-CoA dehydrogenase deficiency

Patient details provided to participants
This young man was evaluated for frequent vomiting and hyperammonemia (118 µmol/L).

Patient details
This patient presented with hyperammonaemia combined with low glutamine (330 µmol/l). The sample was collected during the decompensation. The plasma acylcarnitine pattern was typical of MADD. He did not use any medication apart from acetaminophen and had a normal diet, i.e. no clues for B6 deficiency. No data are available on enzyme testing or genetics, hence the exact diagnosis (ETF, ETFDH or riboflavin metabolism) is not known.

Analytical performance
The following organic acids were mentioned frequently: adipic-, suberic-, sebacic-, glutaric-, 2-OH-glutaric- 3-OH-glutaric-, other 3-OH-dicarboxylic- and 5-OH-hexanoic acid, as well as hexanoyl-, suberyl- isobutyryl- and butyryl-glycine (see Fig. 1 for a typical organic acid chromatogram of the sample). Additionally, a number of labs mentioned elevated 3-OH-butyric acid, but also noted that this was accompanied by a disproportionate dicarboxylic aciduria. Ethylmalonic acid was reported normal. Amino acids and orotic acid were reported as normal, with glutamine being low/decreased. Three participants reported results of acylcarnitine analysis with elevated C0-, C4-, C5- and C5DC-carnitine. Reporting the MADD spectrum of organic acids was marked 2. Reporting only MCADD metabolites was marked 1. Analytical performance was 97%

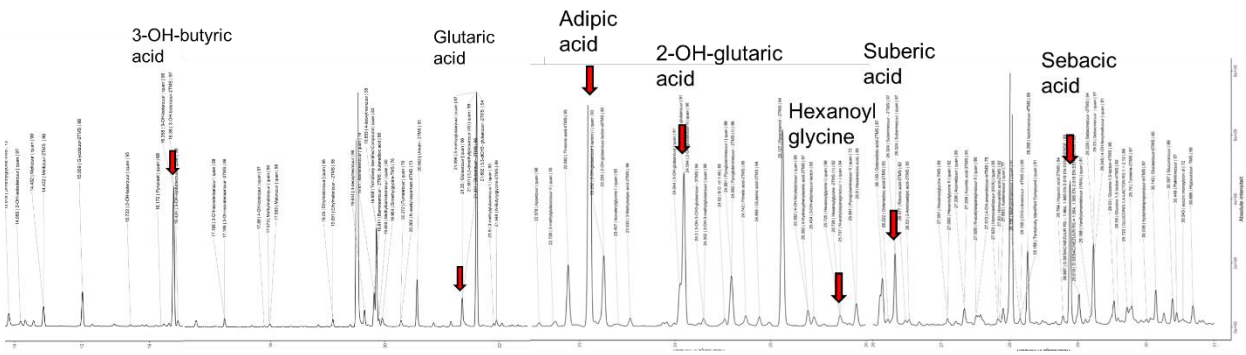

Fig.1. Organic acid chromatogram of sample C.

Diagnosis / Interpretative proficiency
Based on the variety of increased organic acids, dicarboxylic acids and abnormal glycine-conjugates, most participants concluded MADD as a possible diagnosis (14/18; 2 points). Two labs concluded

glutaric aciduria type 1 and two labs reported MCADD as the most likely diagnosis. Reporting GA I or MCADD without mentioning the possibility of MADD was marked 1. A variety of other possible diagnoses were suggested: riboflavin problem, MCT1/SCOT def, ketothiolase def, 2-OH-glutaric aciduria, isobutyryl-CoA DH def, SCADD and ketosis. Interpretative proficiency was 89%.
Presentations of MADD due to ETF(DH) deficiency include (1) a severe neonatal presentation with hypoglycaemia, hyperammonaemia, cardiomyopathy, myopathy, cystic kidney's and dysmorphism; (2) a milder infantile/childhood presentation usually with hypoglycaemia, cardiomyopathy and myopathy. Defects in riboflavin transport or FAD synthesis/transport present in infancy to childhood with cardiomyopathy and myopathy and may biochemically resemble MADD. A particular presentation of riboflavin transport deficiency is Brown-Vialetto-Van Laere, which additionally is characterized by neurodegeneration.

Recommendations
Recommendations included plasma acylcarnitine and amino acid analysis, investigations to determine vitamin B2 and flavins in plasma. Mutation testing was suggested for the ETFA, ETFB, ETFDH genes as well as the genes related to riboflavin transport and metabolism, SLC52A1, SLC52A2, SLC52A3 FLAD1.

Scoring
- Analytical results: reporting abnormal metabolites typical of the MADD spectrum: score 2, reporting only metabolites typical of MCADD: score 1
- Interpretation of results: MADD/GA 2: score 2, suggesting MCADD or GA I WITH the advice to determine plasma acylcarnitines: score 1
- Critical error: failure to report any metabolite typical of MADD. Number of occurrences: 0

Overall impression
Clearly abnormal sample, but detection of dicarboxylic acids and acyl-glycines varied widely between labs. Overall proficiency was 93%.

Multiple distributions of similar samples
In 2019 a MADD sample of a severely affected patient was circulated. Proficiency was 100% for sample 2019-E.

8.5. Patient D – Mucopolysaccharidosis type IIIC (OMIM 252930)

Patient details provided to participants
Boy, 5 y, with coarse facies, thick eyebrows, protruding tongue, low set ears, hyperactivity, speech delay.

Patient details
This patient was initially diagnosed by genetic testing by a homozygous VUS in HGSNAT. Diagnosis MPS IIIC was confirmed by urine GAG analysis.

Analytical performance
Based on the clinical description sample D was an obvious lysosomal storage disease sample and all participants detected increased GAG in this urine sample (1 point), while 16/18 reported elevated heparan sulfate (1 point). The median value of GAG (DMB test) was 22 mg/mmol creat with a rather wide range of 12-41. Increasingly, laboratories report difficulties in purchasing good DMB batches that reliably distinguish normal controls from MPS urine samples. When this remains a problem we advice to validate alternative methods using LC-MS/MS (see below).
Oligosaccharides and sialic acid were normal in the sample.
Whereas several methods have been described 5-10 years ago to analyse GAG by LC-MS/MS, only 3 labs participating in DPT have reported to use such tests (enzymatic GAG hydrolysis with LC-MS/MS of resulting disaccharides, Langereis et al PLoS One 2015 10:e0138622; methanolytic GAG hydrolysis with LC-MS/MS of disaccharides, Zhang et al Mol Genet Metab 2015 114:123-128 and LC-MS/MS of GAG-derived (oligo)saccharides, Saville et al Genet Med 2019 21:753-757). The majority of participants still uses 1-dimensional electrophoresis to perform GAG subfraction analysis. In the case of milder MPS presentations MS-based methods are more sensitive to detect increases of abnormal GAG species (data from the urine MPS scheme). Also LC-MS/MS methods are an alternative for the traditional screening approach using DMB.

Diagnosis / Interpretative proficiency
MPS III was reported by all 16 labs that identified elevated heparan sulfate (2 points). The two remaining participants concluded mucopolysaccharidosis (unspecified) or lysosomal storage disease (either scored with 1 point when appropriate advice for further testing was provided). Some participants still regarded other MPS subtypes as a possibility and mentioned this under 'alternative diagnoses'. With the clear elevation of HS and normal DS, other MPS are unlikely. One lab suggested mucolipidosis type 2/3 as a possibility. With clearly abnormal GAG and normal oligosaccharides, mucolipidosis is less likely than MPS, but mucolipidosis should be included in the differential diagnosis of a possible MPS/oligosaccharidosis patient.

Recommendations
Enzyme (n=12) and genetic (n=15) testing of the 4 different MPS III subtypes was recommended. Two labs advised GAG subtype analysis.

Scoring
- Analytical results: total GAG increased: 1 point, HS elevated (electrophoresis): score 1, increased HS by LC-MS/MS when DMB testing was not performed: score 2
- Interpretation of results: MPS III: score 2, MPS not specified or LSD when appropriate further tests have been suggested: score 1
- Critical error: failure to report increased GAG. Number of occurrences: 0

Overall impression
Obvious LSD sample with clearly abnormal GAG. Overall proficiency (based on scores) 94%.

Multiple distributions of similar samples
Other MPS III samples have been circulated in the DPT-NL scheme in 2011 and 2014. Proficiency was 97 and 67% in these years respectively. Sample 2014-D was obtained from a very mild MPS IIIB patient with slightly elevated GAG, explaining the difficulty to reach the correct diagnosis.

8.6. Patient E – No known IMD diagnosis

Patient details provided to participants
Girl, 5 y. Global developmental delay

Patient details
During metabolic screening no abnormalities were detected in this sample. Apart from metabolic screening and routine cytogenetic testing no further investigations were performed.

Analytical performance
Most participating laboratories performed all tests required for DPT, although some did not investigate purines-pyrimidines and oligosaccharides. In DPT it is allowed to use results of partner labs if this is done on a routine basis. About half of the participants measured additional metabolites not required for DPT participation, such as creatine-guanidinoacetate, sialic acid and acylcarnitines.
Mostly normal results were reported. Three participants reported elevated creatine (median value 889 mmol/mol creat), while 6 other labs considered similar creatine results (median value 824 mmol/mol) as normal. Glyceric acid was reported slightly elevated by 4 labs. Other participants did not mention glyceric acid.
Normal results of GAG screening were reported by 12 laboratories. Three found increased total GAG, from which 2 reported normal results for GAG subtype analysis.

Diagnosis / Interpretative proficiency
No indications for an IMD was concluded by 14 participants. Four labs suggested a diagnosis: creatine transporter defect/carrier status (n=2), glyceric aciduria (n=1) or lysosomal storage disease (n=1).
While a creatine transporter defect is less likely in a girl, it can't be excluded by urine testing. The advice by experts is to perform DNA testing of the SLC6A8 gene for carrier status. A complication in this sample was that creatine was 732 mmol/mol during initial testing in 2011, while the result was 995 mmol/mol upon re-testing in the same laboratory in 2023. Possibly the creatine has increased during storage. During the DPT workshop it was suggested that creatinine may convert to creatine under acidic conditions. The median pH value of sample E was 7.5, however.

The glyceric acid level in the sample was mildly elevated (median value 33 mmol/mol). Literature reports suggest that urine glyceric acid is much higher in patients diagnosed with D-glyceric aciduria due to GLYCTK mutations (Sass et al 2010, Hum Mutat 31:1280-1285). Based on these data glyceric aciduria (GLYCTK deficiency) is less likely. Also, in primary hyperoxaluria type 2 the level of L-glyceric acid is much higher compared to sample 2023-E. Besides, with normal oxalate and absence of symptoms suggestive for nephrocalcinosis, L-glyceric aciduria (PH2) is unlikely.
A lysosomal storage disease (MPS or oligosaccharidosis) is unlikely based on overall results.

Recommendations
The most frequent advice was to complete metabolic workup by tests in plasma or to perform genetic screening. Some specific recommendations were made following up on increased levels of creatine and glyceric acid.

Scoring
- Analytical results: no abnormalities or slightly elevated glyceric acid, creatine or GAG: score 2
- Interpretation of results: no indication for an IMD: score 2, glyceric aciduria or creatine transporter defect/carrier status with appropriate recommendations for further testing: score 2
- Critical error: sample not eligible

Overall impression
The majority of the participants concluded that the results were not suggestive for an IMD. Based on slight abnormalities, some participants suggested diagnoses in this sample from an individual with no known metabolic disorder. Overall proficiency (based on points) was 97%.

Multiple distributions of similar samples
In 2014 sample B was from a patient with no known IMD. Proficiency was 89% for this sample due to 3 labs suggesting (various) incorrect diagnoses. Over-interpretation is known from other DPT circulations; some participants tend to suggest a diagnosis even though the test results are not clearly abnormal.

8.7. Patient F – Methylmalonyl-CoA epimerase deficiency (OMIM 251120) AND sepiapterin reductase deficiency (OMIM 612716).

Patient details provided to participants
Female, born to consanguineous Caucasian parents, evaluated at age 2 for delayed motor development and spasticity. Her motor function gradually deteriorated leading to dystonia. The urine sample was collected at age 21 y while on L-Dopa treatment.

Patient details
This patient with combined methylmalonyl-CoA epimerase deficiency and sepiapterin reductase deficiency was described by Bikker et al, Hum. Mutat. 27: 640-643, 2006 and Abeling et al, Molec. Genet. Metab. 89: 116-120, 2006. Bikker et al. (2006) presented a 16-year-old female patient with persisting moderate methylmalonic aciduria. She was born to consanguineous Caucasian parents originating from the northwest part of the Netherlands. At the age of 2 years, delayed motor development and signs of spasticity were seen. Selective screening for metabolic disease revealed moderate methylmalonic aciduria. Treatment involved reduction of dietary protein, which resulted in significant lowering of urinary methylmalonic acid; however, no clinical effects were observed and the patient's motor function showed a gradual deterioration leading to dystonia. Analysis of pterins and aromatic neurotransmitter metabolites in cerebrospinal fluid at the age of 14 years suggested a defect in sepiapterin reductase, which was subsequently confirmed. Abeling et al. (2006) pointed out a rapid and favourable response on treatment with L-DOPA.

Analytical performance
Elevated methylmalonic acid was reported by 17/18 participants. Many also reported increases in methylcitric acid and 3-OH-propionic acid. Glycine was clearly elevated in this sample with a median value of 973 mmol/mol creat (reported by 16 labs). Increased HVA and VLA were reported frequently and are due to L-DOPA treatment.

Diagnosis / Interpretative proficiency
Defects in mma metabolism including cobalamin defects or (secondary) vitamin B12 deficiency were suggested by 11 labs. Only 2 participants mentioned the possibility of MCEE deficiency. Three participants specifically suggested SUCLG1/SUCLA2 as a diagnosis. Succinyl-carnitine in urine is a

sensitive marker for SUCL defects. Two labs reported values of C4DC-carnitine, 0.7 and 1.2 mmol/mol creat. These values are in the high-normal range. In SUCL urine samples the level is usually 10-fold higher rendering a SUCL defect less likely. A a possible complication of L-DOPA treatment, mentioned by 2 participants, is vitamin B12 deficiency. This may cause increased urinary mma. The mechanism of this side-effect of L-DOPA is unknown.
Two labs reported NKH as a diagnosis. The reason for increased glycine in this sample is unclear. While elevated glycine in plasma and urine is well known in several defects with increased propionyl-CoA (i.e. PA and MMA), this has not been described for MCEE. Although the clinical symptoms are not suggestive for NKH, it can't be excluded based on the analytical results.
In addition, the following diagnoses were suggested frequently: pterin defects/BH4 deficiency, Segawa disease and AADC deficiency. Presumably these were guesses based on clinical symptoms and treatment rather than analytical findings.

Recommendations
Many different suggestions for further investigation were made, including follow-up of the elevated mma.

Scoring
The various abnormalities, not being clearly related to the clinical symptoms and confusing to the participants, made a clear scoring scheme impossible. In view of this, the Scientific Advisory Board has classed sample 2023-F as educational, i.e. it will not be included in performance assessment.

Overall impression
A complicated sample of a patient suffering of two disorders with the clinical symptoms not being related to main analytical findings.

Multiple distributions of similar samples
None

9. Scores of participants

All data transfer, i.e. submission of results as well as viewing and downloading of reports proceed via the DPT-CSCQ results website. The results of participants are confidential and only accessible using username and password on the CSCQ website. Anonymised scores of all laboratories are provided in the annual report. Your results are indicated by an arrow 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 ASL deficiency			Patient B ADSL deficiency			Patient C MADD			
	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	0	0	0	2	2	4	2	2	4	8
5	2	2	4	2	2	4	2	2	4	12
6	2	2	4	2	2	4	2	1	3	11
7	2	2	4	2	2	4	2	2	4	12
8	2	2	4	2	2	4	2	2	4	12
9	0	0	0	0	0	0	2	1	3	3
10	2	2	4	2	2	4	2	2	4	12
11	2	2	4	2	2	4	1	1	2	10
12	2	2	4	0	1	1	2	2	4	9
13	2	2	4	2	2	4	2	2	4	12
14	0	0	0	2	2	4	2	2	4	8
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	2	2	4	2	1	3	11
17	2	2	4	2	2	4	2	2	4	12
18	2	2	4	0	0	0	2	2	4	8

Detailed scores – Round 2

Lab n°	Patient D MPS IIIC			Patient E No IMD			Patient F MCEE + SR			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	--	--	--	8
2	2	2	4	2	2	4	--	--	--	8
3	2	2	4	2	2	4	--	--	--	8
4	2	2	4	2	2	4	--	--	--	8
5	2	2	4	2	2	4	--	--	--	8
6	2	2	4	2	2	4	--	--	--	8
7	2	2	4	2	2	4	--	--	--	8
8	2	2	4	2	2	4	--	--	--	8
9	1	1	2	2	0	2	--	--	--	4
10	2	2	4	2	2	4	--	--	--	8
11	2	2	4	2	2	4	--	--	--	8
12	2	2	4	2	2	4	--	--	--	8
13	1	1	2	2	2	4	--	--	--	6
14	2	2	4	2	2	4	--	--	--	8
15	2	2	4	2	2	4	--	--	--	8
16	2	2	4	2	2	4	--	--	--	8
17	2	2	4	2	2	4	--	--	--	8
18	2	2	4	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	--	20	100	
2	4	4	4	4	4	--	20	100	
3	4	4	4	4	4	--	20	100	
4	0	4	4	4	4	--	16	80	
5	4	4	4	4	4	--	20	100	
6	4	4	3	4	4	--	19	95	
7	4	4	4	4	4	--	20	100	
8	4	4	4	4	4	--	20	100	
9	0	0	3	2	2	--	7	35	
10	4	4	4	4	4	--	20	100	
11	4	4	2	4	4	--	18	90	
12	4	1	4	4	4	--	17	85	
13	4	4	4	2	4	--	18	90	
14	0	4	4	4	4	--	16	80	
15	4	4	4	4	4	--	20	100	
16	4	4	3	4	4	--	19	95	
17	4	4	4	4	4	--	20	100	
18	4	0	4	4	4	--	16	80	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 14 points)	17	94
Unsatisfactory performers (< 14 points and/or critical error)	1	6
Partial and non-submitters	0	0

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-NL-2023-A	ASL deficiency	83	83	83
DPT-NL-2023-B	ADSL deficiency	83	86	85
DPT-NL-2023-C	MADD	97	89	93
DPT-NL-2023-D	MPS IIIC	94	94	94
DPT-NL-2023-E	No IMD	100	94	97
DPT-NL-2023-F	MCEE + SR	--	--	--

10. Annual meeting of participants

The annual DPT meeting was organised online on September 19, 2023 from 9.00 to 10.30. Representatives from many participating labs were present and actively participated in the discussions.

Please note 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 critical review of all results with a discussion on interpretation of results and, if possible, to reach a consensus on best practice.

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

- In 2024 ERNDIM will start a **new pilot scheme, 'Lipids In Serum' (LIS)**, in collaboration with MCA laboratory. This will essentially be a quantitative scheme in which several lipids relevant to IMD diagnostics will be included. Some of the lipids included in LIS will be new, while others have been in the Special Assays Serum scheme for some years already. During the LIS pilot phase, the SAS scheme will not be changed, but when LIS will become a full scheme, some lipids will be removed from SAS.
- **Control materials** are provided by SKML/MCA laboratory since a few years. These are no longer related to EQA materials and have been produced separately. Two concentration levels for each group of analytes are available. The most suitable low and high concentration levels are defined by the scientific advisors of the schemes. Analytes and their concentrations will be similar in consecutive batches of control material. These reference materials can be ordered at MCA laboratory (https://www.erndimqa.nl/) or through the ERNDIM website. Participants are encouraged to use them as internal control samples, but they cannot be used as calibrators. On the ERNDIMQA

website a new section for data management completes the ERNDIM internal Quality Control System. Laboratories have the option to submit results and request reports showing their result in the last run in comparison to defined acceptance limits, their own historical data and the mean of all laboratories using the same batch control material. Control materials for cystine in leukocytes are under development and will probably be available in 2024.

- **Training**:

  In Spring 2024 ERNDIM will organise two workshops, one on amino acids and one on acylcarnitines. These workshops will have the format of a webinar and focus on technical aspects of measuring these two metabolite groups. Dates of these workshops will be announced by email and on the ERNDIM website and registration will be required.

  SSIEM Academy training courses.

  - A 2 days course will be been organized on 22 and 23 April 2024 in Amsterdam. The program includes:
    - Peroxisomal disorders
    - Purine-pyrimidine disorders
    - Lysosomal storage disorders
    - Neurodevelopmental and neurodegenerative disorders
    - Trace elements and metal disorders
  - The lectures will be available on the SSIEM website

- **Urine samples**: To be able to continue this scheme we need a steady supply of new and interesting patient samples. Several laboratories have donated samples in the past, for which they are gratefully acknowledged. If you have one or more samples available and are willing to donate these to the scheme, please contact us at g.ruijter@erasmusmc.nl.

  For the DPT scheme we need at least 300 ml of urine from a patient affected with an established inborn error of metabolism, accompanied by a short clinical report. If possible, please collect 1500 ml of urine: this sample can be used as the common sample and be circulated to all labs participating to the DPT schemes. Each urine sample must be collected from a single patient. Please don't send a pool of urines, except if urine has been collected during a short period of time from the same patient.

  When a donated sample is used, the participating lab donating the sample will have a 20% discount on the DPT scheme fee in the next scheme year.

12. Tentative schedule in 2024

Sample distribution	February 7, 2024
Start of analysis of Survey 2024/1 (website open)	March 4, 2024
Survey 2024/1 - Results submission deadline	April 2, 2024
Survey 2024/1 – Interim report available	April/May 2024
Start of analysis of Survey 2024/2 (website open)	June 3, 2024
Survey 2024/2 – Results submission deadline	June 24, 2024
Survey 2024/2 – Interim report available	July/August 2024
Annual meeting of participants	September 3, 2024 (Porto)
Annual Report 2024	January 2025

13. 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.

14. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the Scientific Advisor of the scheme, George Ruijter (g.ruijter@erasmusmc.nl) and/or to the ERNDIM Administration Office (admin@erndim.org)

Date of report, 2024-01-03

Name and signature of Scientific Advisor

Dr. G.J.G. Ruijter

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

Version Number	Published	Amendments
1	08 January 2024	2023 annual report published

END