ERNDIM Administration Office
c/o EMQN CIC, Unit 4, Enterprise House,
Manchester Science Park, Pencroft Way,
Manchester M15 6SE, United Kingdom
Tel: +44 161 7574952
Fax: +44 161 850 1145
Email: admin@erndim.org

Scientific Coordination
Dr. G.J.G. Ruijter
Erasmus Medical Center
Dep Clinical Genetics
P.O. Box 2040
3000 CA Rotterdam
The Netherlands
Email: g.ruijter@erasmusmc.nl

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: erndim.survey@cscq.ch

Published: 24 March 2025[1]

Diagnostic Proficiency Testing

Centre: The Netherlands

Final Report 2024

prepared by
Dr. G.J.G. Ruijter and Dr. J. Bierau

Note: This annual report is intended for participants of the ERNDIM DPT Netherlands 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 performance of your laboratory, unless ERNDIM is required to disclose performance data by a relevant government agency. For details, please see the terms and conditions in the EQA Schemes Catalogue and Participant Guide and the ERNDIM Privacy Policy on www.erndim.org.

The ERNDIM Diagnostic Proficiency Testing (DPT) Scheme is the ultimate external quality assessment scheme for biochemical genetics laboratories. In 2024, 17 labs participated in the Proficiency Testing Scheme NL.

1. Geographical distribution of participants

For both surveys, all 17 participants have submitted results.

Country	Number of participants
Australia	2
Belgium	5
Czech Republic	1
Germany	1
Netherlands	5
New Zealand	1
South Africa	1
Switzerland	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 and Nicola Braik as scheme organisers (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/

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

Origin of samples: Samples used in 2024 have been provided by:

- AUMC, Amsterdam
- Children's Hospital Zurich
- dr Jasinge, Colombo, Sri Lanka
- dr Mathis, scientific advisor of DPT-CH
- Erasmus MC, Rotterdam

Patient A: Malonic aciduria (common sample provided by DPT-CH)
Patient B: MHBD deficiency
Patient C: Xanthinuria
Patient D: MPS II
Patient E: LPI
Patient F: 2-OH-glutaric aciduria

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 7, 2024: shipment of samples
- March 12, 2024: start analysis of samples of the first survey
- April 2, 2024: deadline for result submission (Survey 1)
- May 7, 2024: interim report with preliminary scores of Survey 1 published
- June 3, 2024: start analysis of samples of the second survey
- June 24, 2024: deadline for result submission (Survey 2)
- August 7, 2024: interim report with preliminary scores of Survey 2 published
- September 3, 2024: DPT participant meeting, Porto
- March 3, 2025: annual report with final scoring published

5. Results

All participants submitted results for both surveys on time.

	Survey 1	Survey 2
Receipt of results	17	17
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:
  - 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 2024 were additionally scored by Dr D. Mathis from DPT CH. At the SAB meeting in Leiden, November 28-29, 2024, 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 2024 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 2024. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

For DPT 2024 a total score of at least 17 points out of the maximum of 24 (71%) and absence of critical errors must be achieved for satisfactory performance.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was generally correct for all labs. One lab had a systematic error in survey one with all three values being too high (treated as outliers). Another lab had an incorrect value in sample 2024-E (also treated as an outlier). Creatinine values are provided in the Table below. CVs are 6-8% for all samples but 2024-B, which had slightly higher CV (14%).

Sample	Median creatinine (mmol/L)	SD (mmol/L)	CV (%)	n
A	8.6	0.5	6.2	16
B	1.8	0.3	14.3	16
C	2.7	0.2	7.4	16
D	7.2	0.5	7.5	17
E	1.5	0.1	6.7	16
F	3.5	0.3	7.7	17

8.2. Patient A – Malonic aciduria due to MLYCD mutations (OMIM 248360)

Patient details provided to participants
Diagnosed by family screening after sudden infant death of brother at 5 months of age in the context of an intercurrent viral infection. Dilated cardiomyopathy, normal development.

Patient details
Cognitive performance at age 15 y is below average, with a mild learning disability. The patient is now integrated in special education. He is overweighted, probably due to treatment with precautionary measures to avoid catabolic state. Treatment includes carnitine substitution.

Sample A was the common sample distributed to participants of all 5 DPT centers and was discussed during the ERNDIM participant meeting in Porto, September 3, 2024, by Dr Deborah Mathis from Bern. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Analytical performance
Elevated malonic acid (reported by 14 labs, median value 31 mmol/mol) or malonyl-carnitine (C3DC; mentioned by 5 labs with median value 5.5 mmol/mol) were the abnormal metabolites in this sample. Either were scored with 2 points. The concentration of malonic acid was relatively low but reflects what has been found in literature (Zhang et al, 2024, Brain & Development 46, 286-293, https://doi.org/10.1016/j.braindev.2024.07.001). Methylmalonic acid (median value 5 mmol/mol) was reported (slightly) elevated by 6 labs, while 4 considered MMA normal and 7 did not mention MMA. Analytical performance was 88%.

Diagnosis / Interpretative proficiency
Malonic aciduria was reported as the most likely diagnosis by 15 labs and fits the clinical synopsis. The function of malonyl-CoA decarboxylase is depicted in figure 1.

One participant considered CMAMMA as the most likely diagnosis, which was reported as alternative diagnosis by another 8 labs. The ratio MMA/MA was used by a number of labs to interpret their analytical findings. In MMA samples the MMA/MA ratio is usually 50-100, while in CMAMMA urine samples this ratio typically is 5-10. Although higher as well as lower values may occur in CMAMMA samples, the ratio will not be lower than 1. In sample 2024 the MMA/MA ratio was 0.16. Interpretative proficiency was 88%.

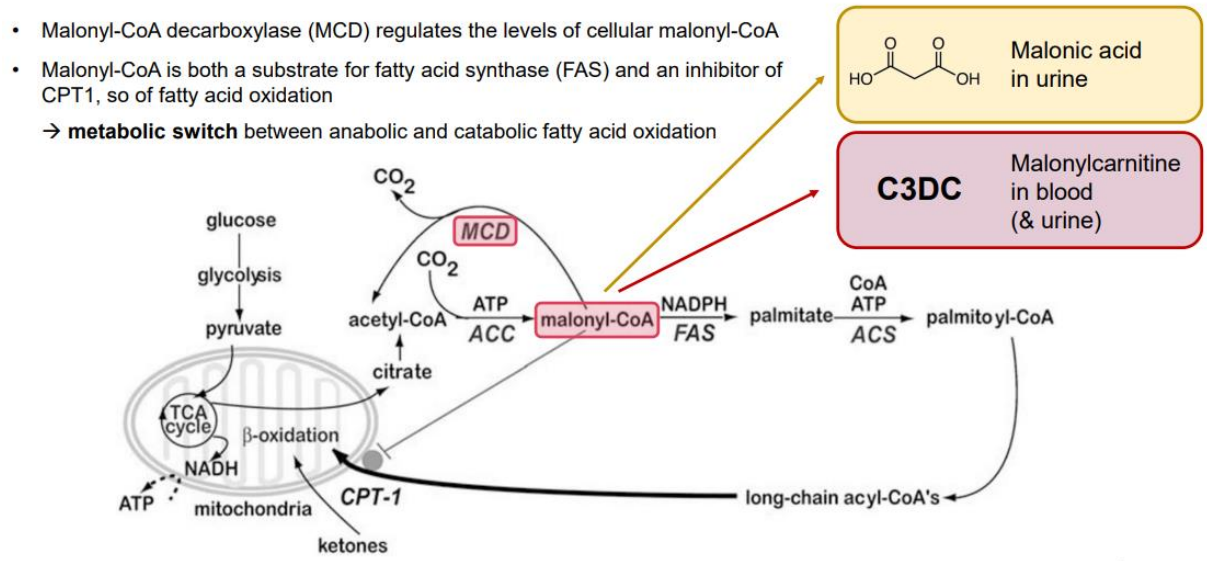

Figure 1. Malonyl-CoA decarboxylase (MCD) function (courtesy of dr D. Mathis).

Recommendations
Advice for further investigations included acylcarnitine analysis in plasma and sequencing of the MLYCD gene. Some participants suggested to analyse MA and MMA in plasma or to measure malonyl-CoA decarboxylase activity in cultured fibroblasts.

Scoring
- Analytical results: elevated malonic acid or malonyl-carnitine: score 2
- Interpretation of results: malonic aciduria as first or alternative diagnosis: score 2, CMAMMA with malonic aciduria not mentioned: score 1
- Critical error: sample not eligible

Overall impression
This was a bit of a challenging sample with moderate malonic acid excretion. Overall proficiency was 88%.

Multiple distributions of similar samples
NA

8.3. Patient B – 2-Methyl-3-OH-butyryl-CoA dehydrogenase deficiency (MHBD def, HSD10 disease, 17-beta-hydroxysteroid dehydrogenase deficiency, OMIM 300438).

Patient details provided to participants
A 4 year-old boy with psychomotor retardation and mild dysmorphic features.

Patient details
This patient was described in Poll-The et al. Mol Genet Metab. (2004) 81:295-299.
Part of the abstract: A 19-month-old boy with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, a defect of isoleucine degradation, had cognitive and motor development delay, spastic diplegia, dysmorphism, and occipital periventricular white matter lesions on MRI scan of the brain. The urinary accumulation of the isoleucine metabolites 2-methyl-3-hydroxybutyrate and tiglylglycine was only moderate under basal conditions. These abnormalities became more pronounced after a 100mg/kg oral isoleucine challenge. Enzyme studies showed a markedly decreased activity of MHBD in fibroblasts and lymphocytes. Sequence analysis of the involved X-chromosome gene (HADH2),

revealed the presence of 364C -->G mutation in the patient. His mother was heterozygous for the 364C-->G mutation, whereas the mutation was not found in the other members of the family (father, brother, and sister).

Analytical performance
Fourteen participants reported elevated tiglylglycine (median value 24 mmol/mol), while ten reported 2-methyl-3-OH-butyric acid (median value 43 mmol/mol). One lab stated that 2-Me-3-OH-butyrate was normal. The levels of these metabolites were only mildly increased and there were large interlaboratory differences in the quantitation of the two components. Tiglylglycine has been part of the ERNDIM quantitative organic acid scheme since its start. Reference values have been reported, 2-Me-3-OH-butyrate 11–27 mmol/mol; tiglylglycine < 3.8 (Poll-The et al. Mol Genet Metab. (2004) 81:295-299, but need to be established from own data. The high sample pH and the presence of nitrite as well as several bacterial metabolites indicates that the sample was contaminated with bacteria, but this did not prohibit diagnosis. Analytical performance was 82%.

Diagnosis / Interpretative proficiency
Quantitation of tiglylglycine was key in this sample and most participants that detected elevated tiglylglycine came to the correct conclusion. In total 13 labs suggested MHBD deficiency (HSD10 disease). Four participants reported 'no diagnosis', but 2 of these suggested MHBD as other possible diagnosis. Other diagnoses mentioned were mitochondrial disorder and sialic acid storage disease. The presence of tiglylglycine should trigger a suspicion of MHBD, in addition to oxothiolase deficiency and propionic acidemia. MHBD can be distinguished from oxothiolase deficiency on the basis of one enantiomer (peak) of 2-methyl-3-hydroxybutyric acid in MHBD and two enantiomers (peaks) in oxthiolase deficiency as well as the presence of 2-methyl-3-oxobutyric acid (2-methyl-acetoacetate) in oxothiolase deficiency. The latter compound is, however, rather unstable and does not survive the pre-treatment that is applied for DPT-samples. Interpretative performance was 79%.

MHBD is an example of a ‘moonlighting’ protein and is identical to the MRPP2 subunit of mitochondrial RNAse P, which is required for tRNA processing. The disease symptoms are currently believed to be mainly related to the RNAse P function. The mitochondrial function may explain the elevated excretion of lactate reported by 7 participants.

Recommendations
Sequence analysis of the HSD17B10 (HADH2) gene, plasma/DBS acylcarnitine testing and repeat analysis of organic acids were suggested most frequently for further testing.

Scoring
- Analytical results: tiglylglycine and/or 2-methyl-3-OH-butyric acid elevated: score 2
- Interpretation of results: MHBD deficiency (HSD10 disease): score 2, mitochondrial disorder with proper recommendations: score 1
- Critical error: sample not eligible

Overall impression
This was a challenging sample with mild abnormalities. Overall proficiency was 81%, which compares quite favorably to the results of the previous circulation of the same sample (2012-B) with overall proficiency 44%.

Multiple distributions of similar samples
Same sample: 2012-B with overall proficiency 44%.

8.4. Patient C – Xanthinuria type I/II

Patient details provided to participants
Girl with urinary stones, currently not under treatment.

Patient details
No further information available.

Analytical performance
All 17 participants reported elevated xanthine and hypoxanthine, while 15 mentioned low/decreased uric acid. The median value for uric acid was 10 mmol/mol while the reference range typically is

around 200-1000 for this age. Normal S-sulfocysteine was reported by 14 labs. Analytical proficiency was 94%.

Diagnosis / Interpretative proficiency
Sixteen participants concluded xanthinuria type I and/or II. Type I is xanthine dehydrogenase (XDH/XO/XOR) deficiency due to mutations in the XDH gene, while type II is a defect in molybdenum cofactor sufuration (MOCOS deficiency) leading to lack of XDH and aldehyde oxidase (AO) activity (figure 2). The exact diagnosis was not known at the time the sample was circulated. Six participants specifically mentioned that the 2 types can't be distinguished based on clinical symptoms or urine testing. One participant concluded HPRT deficiency. Six labs stated that MoCo deficiency was excluded or unlikely based on normal S-sulfocysteine. Interpretative proficiency was 94%.

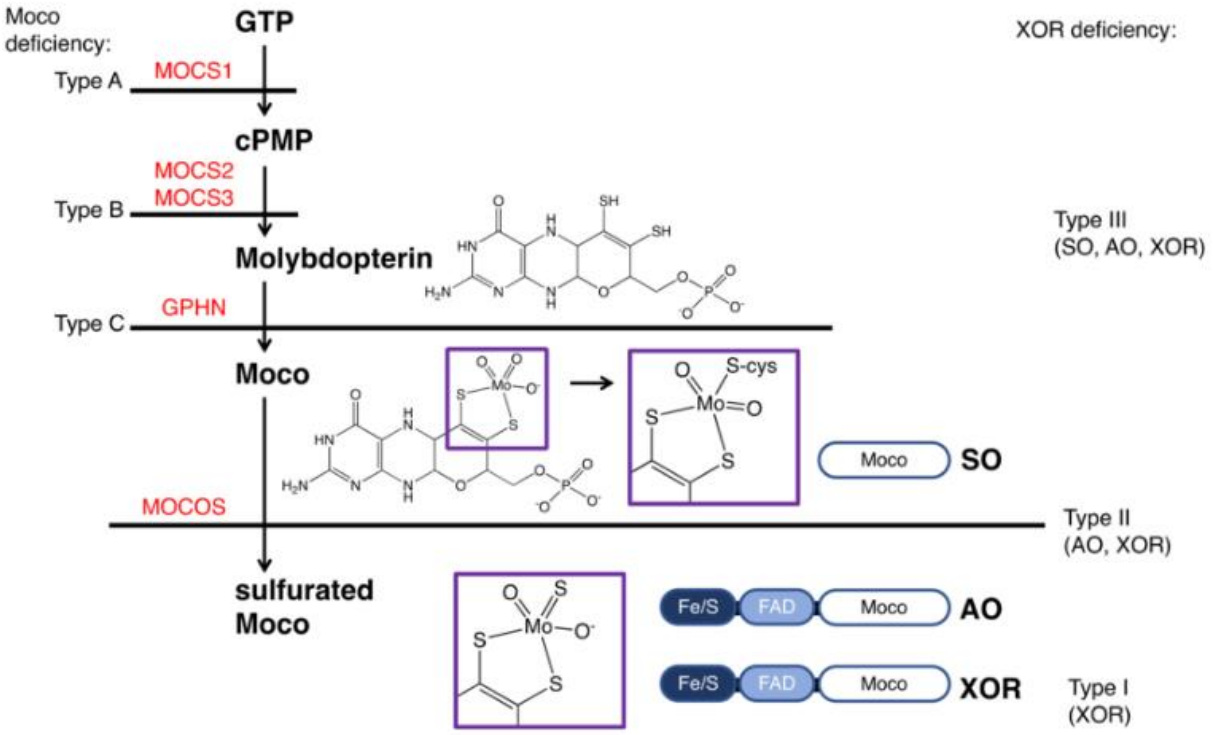

Figure 2. MoCo biosynthesis. XOR, xanthine oxidase; AO, aldehyde oxidase.

Recommendations
Recommendations included sequencing of the XDH and MOCOS genes (almost all labs) and to perform an allopurinol loading test to distinguish type I and II. The usefulness of this loading test is explained as follows. Conversion of Allopurinol to Oxypurinol can be catalysed by XDH (XOR) as well as AO. In the case of XDH deficiency this conversion can still be performed by AO, while MOCOS deficiency blocks both enzymes. In the latter case Oxypurinol will not be produced.

Metabolites specific to xanthinuria type II would help to distinguish type I and II. Several such metabolites are known, but typically are not included in the tests performed by IMD laboratories. In figure 3 the involvement of aldehyde oxidase in various metabolic pathways and the corresponding metabolites relevant to diagnostic testing are shown (courtesy of dr Leo Kluijtmans). One participant in fact suggested to measure N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-5-carboxamide, which should be low in a MOCOS deficiency and normal in a XDH deficiency. Coene et al (J Inherit Metab Dis. 2028, 41:337-353) have shown the ability to establish a precise diagnosis of xanthinuria type II based on results of untargeted metabolomics in plasma.

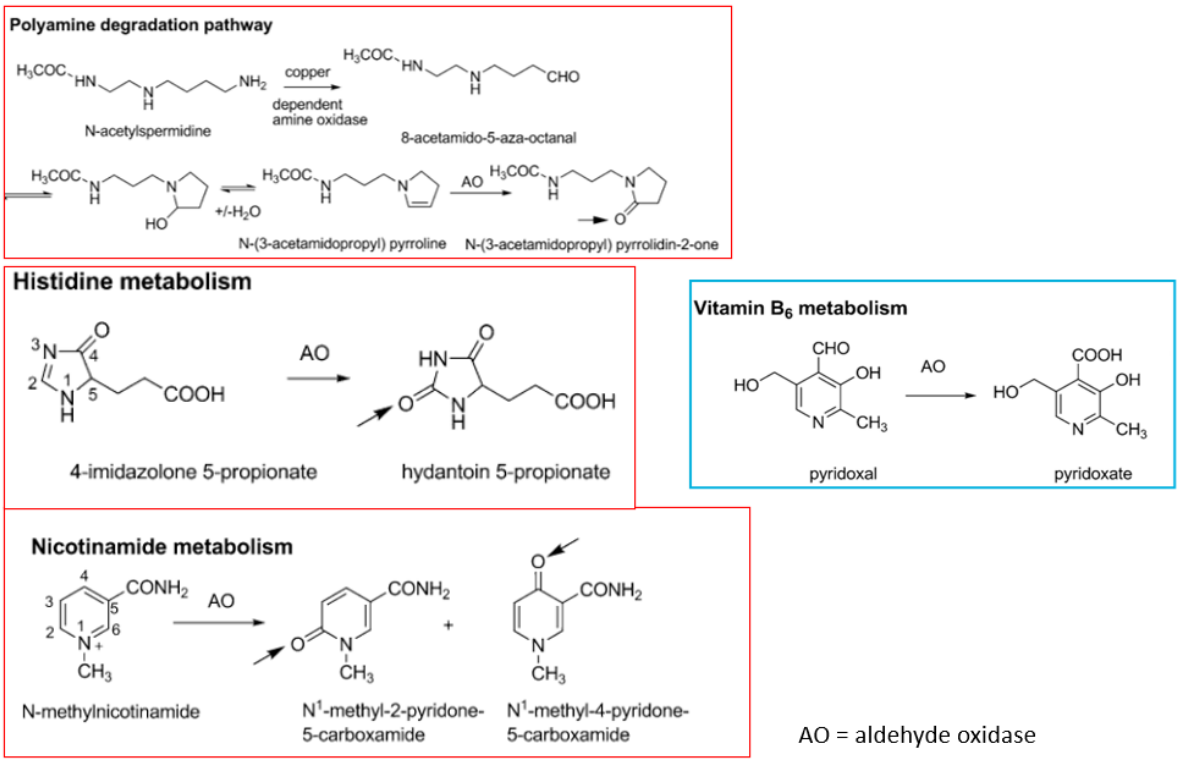

Figure 3. Involvement of aldehyde oxidase in various metabolic pathways (courtesy of dr Leo Kluijtmans).

Scoring

- Analytical results: xanthine and hypoxanthine elevated and uric acid low/decreased: score 2, xanthine and hypoxanthine elevated and uric acid normal or not mentioned: score 1
- Interpretation of results: xanthinuria: score 2
- Critical error: no abnormalities and incorrect/no diagnosis (n=0)

Overall impression

Obvious xanthinuria sample with high overall proficiency: 94%.

Multiple distributions of similar samples

NA

8.5. Patient D – Mucopolysaccharidosis type II (Hunter syndrome; OMIM 309900)

Patient details provided to participants

A male patient diagnosed at age 7 y with stiff joints and claw hands. The urine sample was obtained at age 45 y.

Patient details

Diagnosis was confirmed by iduronate sulfatase deficiency and an IDS mutation. Patient is not on ERT.

Analytical performance

Increased GAG excretion was reported by all 17 labs. Elevated DS was specifically reported by 14 participants, while 12 reported increased HS excretion. Increased CS was reported by 2 labs. Four labs (possibly 5; methods are not always clearly decribed by participants) used LC-MS/MS to investigate GAGs and reported elevated DS+HS or MPS II-specific oligosaccharides. Literature references describing LC-MS/MS analysis of GAG are: 1. Langereis et al PLoS One 2015 10:e0138622 (enzymatic GAG hydrolysis, followed by LC-MS/MS of disaccharides), 2. Zhang et al Mol Genet Metab 2015 114:123-128 (methanolytic GAG hydrolysis, followed by LC-MS/MS of

disaccharides) and 3. Saville et al Genet Med 2019 21:753-757(LC-MS/MS of GAG-derived non-reducing end (oligo)saccharides derivatised by PMP).
Oligosaccharides and sialic acid were normal. Analytical performance was 100%.

Diagnosis / Interpretative proficiency
A number of different combinations of MPS were reported (including I, II, I/II, I/II/VII, VI) for most likely diagnosis. As expected, based on the analytical results most of the labs (15) mentioned MPS II as the most likely or alternative diagnosis (score 2). Two participants concluded MPS I for this sample without mentioning MPS II as a possibility (score 1). This shows that with most of the methods currently used to test for MPS an exact diagnosis, i.e., distinguishing MPS I and II is not possible. The methods using non-reducing end (sulfated) sugars and oligosaccharides appear superior and able to distinguish all MPS types.
Mucolipidosis (type 2/3) and multiple sufatase deficiency were mentioned as other possible diagnosis. Mucolipidosis seems less likely, since in mucolipidosis type 2 abnormalities in oligosaccharides are expected, while in mucolipidosis type 3, GAG are usually not strongly abnormal. Multiple sulfatase deficiency is very rare; it is not clear whether mild presentations exist which show elevated GAG in urine. A new group of defects have recently been described: MPS-plus syndromes with defects in the HOPS and CORVET complexes which are involved in endocytosis (vesicle transport and fusion to the lysosome). Examples of MPS-plus syndromes include defects in VPS33A and VPS16. Again, it is not clear whether mild presentations exist which show elevated GAG in urine.
Interpretative performance was 94%.

Recommendations
All participants advised to perform enzyme activity testing and molecular testing on IDUA/IDS.

Scoring
- Analytical results: Elevated total GAG, established by e.g., the DMB-test: score 1. Abnormal GAG results in electrophoresis/TLC: score 1. MS tests: elevated DS+HS or MPS II-specific oligosaccharides: score 2
- Interpretation of results: MPS II included in DD: score 2, Mucopolysaccharidosis unspecified or wrong type MPS: score 1
- Critical error: failure to report abnormal GAG or MPS (n=0)

Overall impression
Straightforward MPS sample with high proficiency (97%).

Multiple distributions of similar samples
In 2015 sample A was obtained from another adult MPS II patient, with comparable overall proficiency (90%).

8.6. Patient E – Lysinuric Protein Intolerance (LPI, OMIM 222700)

Patient details provided to participants
A 4-year old boy referred for vomiting and refusal to eat. He had multiple bone fractures in the past years.

Patient details
First plasma amino acid results were (all umol/L): lysine 21, arginine 7, ornithine 8, glutamine 1731, alanine 943. Blood ammonia was 53 umol/L. The diagnosis LPI was confirmed by two pathogenic mutations in SLC7A7.

Analytical performance
Most labs (15/17) reported elevated lysine, arginine and ornithine. One lab reported normal lysine and another normal arginine, which may be related to incorrect reference values as all 3 amino acids were clearly elevated. Orotic acid was reported elevated by 14 participants and several also mentioned elevated uracil. Orotic acid was only slightly elevated (median value 10 mmol/mol), which fits the value of blood ammonia (53 umol/L). Analytical proficiency was 85%.

Diagnosis / Interpretative proficiency
Interpretation of the amino acid pattern was not completely straightforward, since besides lysine/arginine/ornithine several other amino acids were slightly elevated (e.g. glycine and alanine),

but not to the extent observed for lysine, arginine and ornithine. Increased orotic acid and/or uracil was helpful and 15/17 labs concluded LPI, while one mentioned LPI as the alternative diagnosis. HHH syndrome and other UCD's were suggested as alternative diagnoses by 9 participants. Homocitrulline (median 24 mmol/mol, n=3) did not appear to be clearly elevated. Hypophosphatasia was also stated as a possible diagnosis. The excretion of phosphoethanolamine was normal though (21 mmol/mol).
Interpretative proficiency was 91%.

Recommendations
The following recommendations were made: analysis of plasma amino acids, blood ammonia and SLC7A7 mutation testing. Several labs suggested analysis of ferritin and LDH..

Scoring
- Analytical results: elevated lysine and arginine and ornithine: score 1, elevated orotic acid: score 1
- Interpretation of results: LPI as most likely diagnosis: score 2, incorrect first diagnosis with LPI given as alternative diagnosis: score 1
- Critical error: failure to report LPI as a possible diagnosis (n=1)

Overall impression
Lysine, arginine and ornithine were clearly abnormal (5-10 fold upper limit of reference values) and this should be noticed and reported along with the possibility of LPI, for which treatment options are available.
Overall proficiency of this sample was reasonable (88%).

Multiple distributions of similar samples
Sample 2017-F was a slightly different LPI sample with similar lysine and orotic acid levels, but lower concentrations of arginine and ornithine. This possibly explains the somewhat lower overall proficiency in 2017 (79%).

8.7. Patient F – 2-OH-glutaric aciduria

Patient details provided to participants
An 11 year-old boy with attention deficit hyperactive disorder. He gradually developed cerebellar signs (intentional tremors, ataxic gait).

Patient details
This child was diagnosed as having attention deficit hyperactive disorder and was seen by a psychiatrist. Gradually he developed cerebellar signs (intentional tremors, ataxic gait) and the neurologist wanted to exclude juvenile Canavan disease. Organic acid profile instead revealed high 2-hydroxy-glutaric acid. The diagnosis was genetically confirmed with two mutations in the L2HGDH gene.

Analytical performance
Strongly increased excretion of 2-OH-glutaric acid was detected by all 17 participants. Two participants performed separation of D- and L-2-OH-glutaric acid and reported a predominant increase in L-2-OH-glutaric acid. Just one laboratory mentioned normal glycine conjugates (hexanoyl-, suberyl-, phenylpropionylglycine) and ethylmalonic acid, while 2 labs reported normal glutaric acid.

Diagnosis / Interpretative proficiency
Diagnostic proficiency was excellent: all 17 participants concluded 2-OH-glutaric aciduria. 14/20 reported L-2-OH-glutaric aciduria as the most likely diagnosis, generally based on the clinical symptoms. The possibility of D- or combined D-/L-2-OH-glutaric aciduria was mentioned by almost all participants as an alternative diagnosis. Two labs noted that MADD was possible, but unlikely with the clinical information provided. The absence of other abnormalities in the organic acid profile also rules out MADD.

Recommendations
Many participants advised to perform separation of the 2-OH-glutaric enantiomers in another specialized laboratory. All labs advised to perform mutation analysis of 2 or more of the L2HGDH, D2HGDH, IDH1, IDH2 and SLC25A1 genes.

Scoring

- Analytical results: elevated 2-OH-glutaric acid: score 2
- Interpretation of results: 2-OH-glutaric aciduria with appropriate advice for molecular testing: score 2
- Critical error: failure to report 2-OH-glutaric aciduria (n=0)

Overall impression

Easy sample with overall proficiency 100%.

Multiple distributions of similar samples

Sample 2011-A was a different L-2-OH-glutaric aciduria sample with overall proficiency 94%.
Sample 2017-B was a D-2-OH-glutaric aciduria sample, due to IDH2 mutations, with overall proficiency 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.

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 Malonic aciduria			Patient B MHBD deficiency			Patient C Xanthinuria			
	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	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	2	2	4	0	0	0	1	2	3	7
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	0	0	0	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	0	0	0	0	0	0	1	0	1	1
14	2	2	4	2	2	4	2	2	4	12
15	0	0	0	2	1	3	2	2	4	7
16	2	2	4	2	2	4	2	2	4	12
17	2	2	4	2	2	4	2	2	4	12

Detailed scores – Round 2

Lab n°	Patient D MPS II			Patient E LPI			Patient F 2-OH-glutaric aciduria			
	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	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	1	3	2	2	4	2	2	4	11
8	1	1	2	2	2	4	2	2	4	10
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	1	2	3	2	2	4	11
12	2	2	4	2	2	4	2	2	4	12
13	2	2	4	0	0	0	2	2	4	8
14	2	2	4	1	2	3	2	2	4	11
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	1	1	2	2	2	4	10
17	2	2	4	2	2	4	2	2	4	12

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	4	4	4	4	4	24	100	
4	4	4	4	4	4	4	24	100	
5	4	4	4	4	4	4	24	100	
6	4	4	4	4	4	4	24	100	
7	4	4	4	3	4	4	23	96	
8	4	0	3	2	4	4	17	71	
9	4	4	4	4	4	4	24	100	
10	4	0	4	4	4	4	20	83	
11	4	4	4	4	3	4	23	96	
12	4	4	4	4	4	4	24	100	
13	0	0	1	4	0	4	9	38	CE
14	4	4	4	4	3	4	23	96	
15	0	3	4	4	4	4	19	79	
16	4	4	4	4	2	4	22	92	
17	4	4	4	4	4	4	24	100	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 17 points	16	94
Unsatisfactory performers (< 17 points and/or critical error)	1	6
Partial and non-submitters	0	0

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-NL-2024-A	Malonic aciduria	88	88	88
DPT-NL-2024-B	MHBD deficiency	82	79	81
DPT-NL-2024-C	Xanthinuria	94	94	94
DPT-NL-2024-D	MPS II	97	94	96
DPT-NL-2024-E	LPI	85	91	88
DPT-NL-2024-F	2-OH-glutaric aciduria	100	100	100

10. Annual meeting of participants

The annual DPT meeting was organised in Porto on September 3, 2024, 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 has started 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 is available for testing purposes.

- **Training**:

  In Spring 2024 ERNDIM organised two webinars, one on amino acids and one on acylcarnitines, focusing on technical aspects of measuring these two metabolite groups. In 2025 ERNDIM plan to organise webinars on organic acids and purines-pyrimidines. 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 day course will be organised on the 28th and 29th April 2025 in Prague. The program includes:
  - Mitochondrial disorders
  - Glycogen storage disorders
  - Neurotransmitter disorders
  - Congenital disorders of glycosylation

- **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. Reminders

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

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides
- Purine/pyrimidines

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 in 2025

Sample distribution	February 5, 2025
Start of analysis of Survey 2025/1 (website open)	March 17, 2025
Survey 2025/1 - Results submission deadline	April 7, 2025
Survey 2025/1 – Interim report available	May 2025
Start of analysis of Survey 2025/2 (website open)	June 2, 2025
Survey 2025/2 – Results submission deadline	June 23, 2025
Survey 2025/2 – Interim report available	July/August 2025
Annual meeting of participants	October 2025
Annual Report 2025	January 2026

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, George Ruijter (g.ruijter@erasmusmc.nl) and/or to the ERNDIM Administration Office (admin@erndim.org).

Date of report, 2025-03-24

Name and signature of Scientific Advisor

Dr. G.J.G. Ruijter
Erasmus Medical Center
Dep Clinical Genetics
P.O. Box 2040
3000 CA Rotterdam
The Netherlands
Email: g.ruijter@erasmusmc.nl

APPENDIX 1 Change log (changes since the last version)

Version Number	Published	Amendments
1	24 March 2025	2024 annual report published

END