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Diagnostic Proficiency Testing

Centre: The Netherlands

Final Report 2022

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 2022, 18 labs participated in the Proficiency Testing Scheme NL.

1. Geographical distribution of participants

17 participants submitted reports for both surveys, while one participant submitted results for survey 1 only.

Country	Number of participants
Australia	2
Belgium	5
France	1
Germany	1
Netherlands	6
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 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 2022 have been provided by:

- dr Songailiene, Vilnius, Lithuania
- dr Jasinge, Colombo, Sri Lanka
- dr Vianey-Saban, scientific advisor of DPT-F
- AUMC, Amsterdam
- One sample was obtained with help of VKS, the Dutch patient organization

Patient A: Barth syndrome ; common sample provided by DPT UK
Patient B: I-cell disease
Patient C: Hyperprolinemia type II
Patient D: CTX
Patient E: MCADD
Patient F: GS deficiency

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.
Mailing: 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 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 neighboring 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 2, 2022: shipment of samples
- March 14, 2022: start analysis of samples of the first survey
- April 4, 2022: deadline for result submission (Survey 1)
- May 13, 2022: interim report with preliminary scores of Survey 1 published
- June 6, 2022: start analysis of samples of the second survey
- June 28, 2022: deadline for result submission (Survey 2)
- July 19, 2022: interim report with preliminary scores of Survey 2 published
- August 30, 2022: DPT workshop in Freiburg
- January 17, 2023: annual report with final scoring published

5. Results

One participant submitted results for survey 2022-1 only. All other participants submitted results for both surveys on time.

	Survey 1	Survey 2
Receipt of results	18	17
No results submitted	0	1

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 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 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. Performance is evaluated only for laboratories submitting results for both surveys.

Scoring and certificate of participation
Scoring is carried out by the scientific advisor as well as a second assessor from another DPT scheme who changes every year. The results of DPT NL 2022 have been scored additionally by Dr Joanne Croft, from DPT-UK. At the SAB meeting in Rome, November 24-25, 2022, 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 2022 samples are given under 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. Two performance support letters will be sent by the Scheme Advisor for 2022. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

As announced in the annual report of the 2021 scheme year the minimum score for satisfactory performance is raised from 62% to 70% starting in the scheme year 2022. Sample 2022-D (CTX) was classed educational and will not be scored. 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.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was mostly correct for all labs. Three clearly incorrect values were noticeable, probably clerical errors, but no systematic errors were present. The 3 outliers were removed from the dataset. Creatinine values are depicted in the table below. CV's are 6-10%.

Sample	Median creatinine (mmol/L)	SD (mmol/L)	CV (%)	n
A	2,1	0,17	8,1	17
B	7,75	0,5	6,5	16
C	1,5	0,12	8,0	16
D	7,2	0,65	9,0	17
E	4,9	0,33	6,7	17
F	2,3	0,2	8,7	17

8.2. Patient A – Barth syndrome (3-methylglutaconic aciduria type II, OMIM 302060) due to mutations in TAFAZZIN

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

Patient details
Patient with pre-natal growth concerns. He was monitored throughout life for poor growth (height and weight < 2nd centile). He presented at 4 years of age due to lips going blue during exercise and was found to have cardiomyopathy. He was diagnosed at 14 years when genome sequencing became available. The diagnosis was confirmed by bloodspot cardiolipin analysis. Barth syndrome is a secondary 3-methylglutaconic aciduria due to defective phospholipid remodelling.
Sample A was the common sample distributed to participants of all 5 DPT centers and was discussed during the ERNDIM participant meeting in Freiburg, August 30, 2022 by dr Claire Hart from Sheffield. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Analytical performance
Elevated 3-methylglutaconic acid, the key compound to reach diagnosis in this sample, was reported by all 18 participants, while 13 mentioned an abnormal value of 3-methylglutaric acid. Various other abnormalities in organic acid excretion, such as lactate, 3-OH-isovaleric acid, 2-ethylhydracrylic acid and succinic acid, were reported which reflected the condition of the patient at the time of urine collection. The 3-methylglutaconic acid (3MGA) concentration was not very high (median value 50 mmol/mol). When quantitative analysis is performed on this sample, the result is clearly higher than the reference values (commonly 0-20 mmol/mol). 3-Methylglutaconic acid is commercially available (e.g. Sigma-Aldrich) to use as a calibrator.
Analytical proficiency was 100%.

Diagnosis / Interpretative proficiency
Sixteen participants concluded Barth syndrome (3MGA-uria type II) as the most likely diagnosis and 2 participants concluded secondary 3MGA-uria. Both were scored with 2 points as mild/secondary 3MGA-uria is observed in several different IEM and more investigations are required to establish the precise diagnosis. The specific type of secondary 3MGA-uria in sample 2022-A was most probably concluded by most participants on the basis of the clinical symptoms.
Alternative diagnoses provided included other secondary 3MGA-uria and mitopathy. One participant regarded primary 3MGA-uria possible, but 5 others stated that this was unlikely because (1) the 3MGA concentration was not in the range expected for primary 3MGA-uria, (2) the ratio 3MGA/3-methylglutaric acid was <20, and (3) 3-OH-isovaleric acid was not clearly elevated. Iminoglycinuria was mentioned by 1 participant. Several amino acids were indeed slightly elevated. This might be due to tubulopathy.
Diagnostic proficiency was 100%.

Recommendations
Recommendation suggested included TAZ gene testing, cardiolipin analysis (mainly in DBS), mutation testing of other genes associated with secondary 3MGA-uria, neutropenia investigations,repeat organic acid analysis, lactate and pyruvate determination in blood and plasma acylcarnitines.

Scoring
- Analytical results: elevated 3-methylglutaconic acid: score 2
- Interpretation: Barth syndrome/3MGA-uria type II or secondary 3MGA-uria: score 2
- Critical error: not reporting elevated 3MGA. Number of occurrences: 0

Overall impression
Excellent overall proficiency (100%) indicates that all participating laboratories are well aware of moderate 3MGA increases.

Multiple distributions of similar samples
In 2018 another Barth syndrome sample was circulated with overall proficiency 86%.

8.3. Patient B – I-cell disease (mucolipidosis type II, OMIM 252500) due to mutations in GNPTAB

Patient details provided to participants
2-Year-old boy investigated for coarse facies, dental hyperplasia, hydrocephaly, short neck, deformation of chest, contractures of joints and mild hypotonia.

Patient details
This boy presented with symptoms typical for a lysosomal storage disorder. He was diagnosed with I-cell disease based on strongly elevated levels of lysosomal enzymes in plasma. In I-cell disease targeting of lysosomal enzymes to the lysosome is defective due to absent/decreased activity of the GlcNAc-phosphotransferase encoded by the GNPTAB and GNPTG genes. This results in excretion of lysosomal enzymes by cells explaining the high enzyme levels in plasma. Cells have deficiencies of many lysosomal enzymes and this can be used in diagnostic testing with fibroblasts. In leucocytes deficiencies are less pronounced.

Analytical performance
Variable results regarding LSD investigations were reported for this sample. Most consistent was abnormal oligosaccharides, reported by 14 participants. Two labs found a normal oligosaccharide pattern and 2 did not report about oligosaccharides. The pattern observed in this urine sample resembles that of sialidosis (Fig. 1). Seven labs reported elevated total GAGs, but 9 labs a normal level. Some labs mentioned that the increase in total GAG was mild or borderline. GAG subtype analysis was reported abnormal by 7 labs, with mildly abnormal heparan sulphate and/or dermatan sulphate. GAG subtype analysis was reported normal by 3 labs. Abnormal oligosaccharides was scored 2, abnormal GAG was marked 1.
Analytical proficiency was 89%.

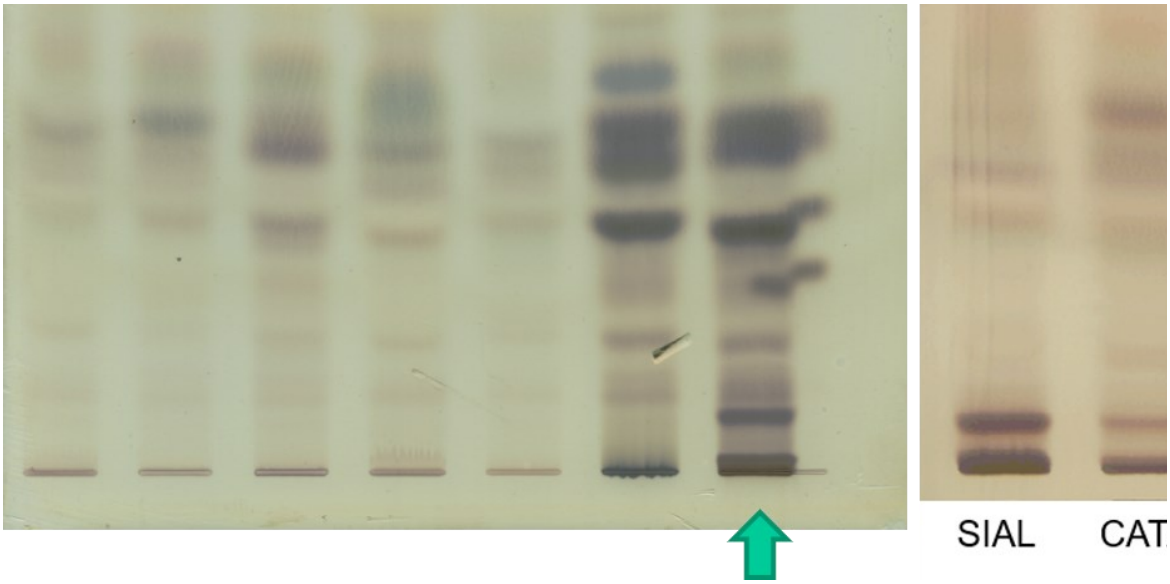

Figure 1. Oligosaccharide TLC of sample B (green arrow).

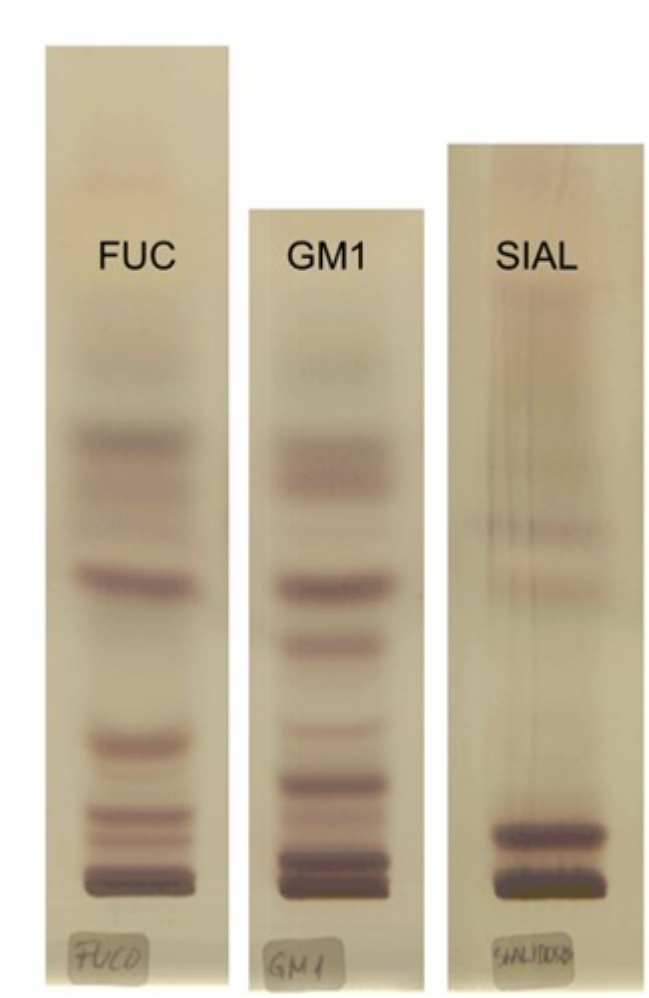

Figure 2. Oligosaccharide TLC patterns of fucosidosis, GM1 and sialidosis.

Diagnosis / Interpretative proficiency

The diagnoses submitted reflected the analytical results. Only 7 participants mentioned I-cell disease/mucolipidosis type II as the most likely or other possible diagnosis (score 2). Other diagnoses mentioned were various MPS (9 participants) and specific oligosaccharidoses (4 labs), all scored with

1 point. With regard to interpretation of TLC oligosaccharides, the pattern observed is different from GM1 gangliosidosis (Fig. 2).
The educational value of sample B is: when abnormal oligosaccharides are found, resembling a sialidosis pattern, with normal or slightly abnormal GAG, then include mucolipidosis type II in the differential diagnosis. Literature and text books on ML II report that oligosaccharides are clearly abnormal, while GAG can be normal or slightly abnormal. (e.g. Physician's guide to the diagnosis, treatment and follow-up of Inherited Metabolic Diseases, Blau et al eds, 2022, chapter 'Mucolipidoses, Multiple Sulfatase Deficiency, and Cathepsin K and C Deficiency', Hidde H. Huidekoper, Esmee Oussoren, pages 1235-1247). Note that hypertrophy of the gums ('gingival hypertrophy') is a clinical symptom suggesting mucolipidosis type II.
Interpretative proficiency was 69%.

Recommendations
Suggestions for further investigations specific for I-cell disease were: measurement of lysosomal enzyme acivities in plasma and fibroblasts and GNPTAB mutation testing. Other recommendations provided were: repeat oligosaccharide analysis and testing of (specific) lysosomal enzymes/genes.

Scoring
- Analytical results: abnormal oligosaccharides: score 2; abnormal GAG: score 1
- Interpretation of results: I-cell disease/mucolipidosis type II mentioned as the most likely or other possible diagnosis: score 2; any oligosaccharidosis or mucopolysaccharidosis: score 1
- Critical error: no potential critical errors were identified for this sample

Overall impression
Overall proficiency (based on points) was 79%.

8.4. Patient C - Hyperprolinemia type II due to delta- 1-pyrroline-5-carboxylate (P5C) dehydrogenase (OMIM 239510)

Patient details provided to participants
Girl, presenting at 4 years of age with speech delay, hyperactive behaviour and suspicion of autism. EEG was normal. Urine sample was collected at age 7.

Patient details
Hyperprolinaemia type II was suspected at 4 years of age because of a strong increase of plasma proline (2350 µmol/l), with an increase of plasma pyrroline-5-carboxylic acid (evaluated at 6 µmol/L by LC/MS-MS). Proline was also highly increased in urine (1059 mmol/mol creatinine), with an increase of pyrroline-5-carboxylic acid (evaluated at 63 mmol/mol creat by LC/MS-MS). Moreover N-(pyrrole-2-carboxyl) glycine was identified in her urinary organic acid profile. The diagnosis was confirmed by mutation analysis of ALDH4A1 gene.
Hyperprolinaemia type II is caused by a deficiency of pyrroline-5-carboxylate dehydrogenase (P5CDH), a mitochondrial inner-membrane enzyme which converts pyrroline-5-carboxylic acid into glutamic acid. Proline metabolism and the P5C dehydrogenase defect is shown in Fig. 3.
Hyperprolinaemia type II is generally associated with epilepsy and mental retardation, but asymptomatic patients have been described. Pyrroline-5-carboxylic acid (P5C), which accumulates in this disease, is an antagonist of (reacts with) vitamin B6 (pyridoxine), and seizures can be due in part to B6 inactivation. In literature there is controversy as to whether seizures are B6 responsive.
Identification of P5C or its metabolites (e.g. N-(pyrroline-2carboxyl)-glycine) allows differentiation between type II and type I hyperprolinaemia. Plasma proline levels are higher in hyperprolinaemia type II (usually > 2000 µmol/L) than in type I.

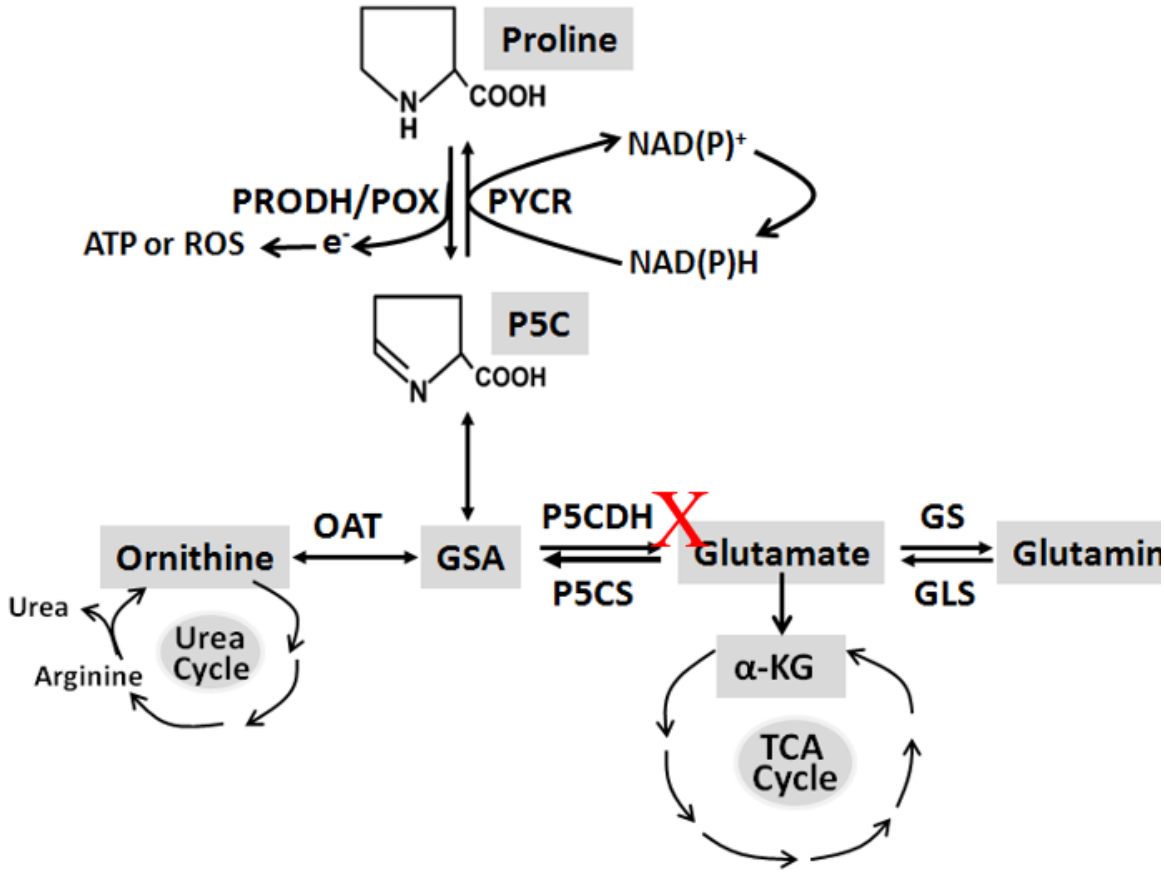

Figure 3. Proline metabolism.

Analytical performance

All labs, except one, reported elevated proline (marked 1). Many labs also reported elevations of glycine and hydroxy-proline (Biochrom chromatogram shown in Fig. 4), which is probably due to decreased renal re-absorption and results from competion by high proline for the common transporter. Some participants reported increased lysine an ornithine. The latter may be formed from accumulating P5C by OAT (Fig. 3).

Elevated N-(pyrrol-2-carboxyl)glycine) in organic acid analysis was mentioned by 11 participants, while two reported increased pyrroline-5-carboxylic acid (either marked 1). The organic acid chromatogram obtained in the scientific advisor's laboratory is shown in Fig. 5, while the mass spectra of the mono-, di- and tri-TMS derivatives of N-(pyrrol-2-carboxyl)glycine are depicted in Fig. 6. The patient was treated with pyridoxine as shown by the high concentration of a B6-derivative in the organic acid chromatogram. Analytical proficiency was 78%.

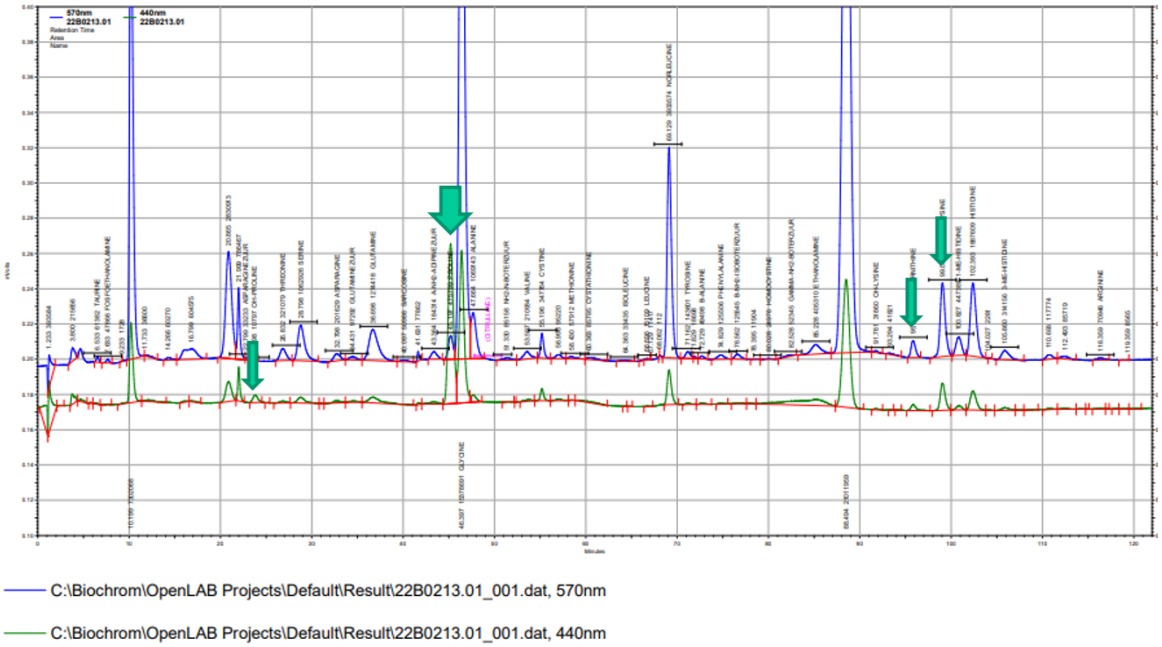

Figure 4. Amino acid analysis sample C using Biochrom.

Diagnosis / Interpretative proficiency
Hyperprolinemia type II (as a most likely or other possible diagnosis) was reported by 12 participants. Other diagnoses mentioned were iminoglycinuria (n=2), hyperprolinemia type I (n=2), AGAT (n=1) deficiency, MPS III (n=1) and 'no diagnosis' (n=1). Hyperprolinemia type I and iminoglycinuria are incorrect, but scored with 1 point. Both diagnoses could be discarded when P5C or its metabolites were identified. The urine amino acid pattern cannot discriminate between hyperprolinemia type I or II or iminoglycinuria. AGAT deficiency is unlikely based on the normal excretion of guanidinoacetic acid (median value: 28 mmol/mol). Also MPS III is unlikely; total GAG was reported normal by 6 participants, but increased by 3 others.
Interpretive proficiency was 75%.

Recommendations
Recommendations provided and related to the correct diagnosis were: analysis of plasma amino acids (n=5), ALDH4A1 mutation testing (n=9), determination of P5C (n=3) and P5CDH activity testing (n=2).

Scoring

- Analytical performance: increase of proline (score 1); increase of N-(pyrrole-2-carboxyl) glycine or N-pyrrole-2-carboxylic acid or pyrroline-5-carboxylic acid (score 1)
- Interpretation of results: hyperprolinaemia type II as most likely or other possible diagnosis; hyperprolinaemia type I or iminoglycinuria: score 1
- Critical eror: the SAB has considered as a critical error the failure to detect an increase of proline in this sample, because all but 1 participant detected it and the possibility to treat the patient with B6. Number of occurrences: 1.

Overall impression
Two thirds of the participating labs identified pyrrol-carboxylglycine or P5C and reached the correct diagnosis. Overall proficiency was 76%. Diagnosing hyperprolinemia type 2 is important for treatment of possible vitamin B6 deficiency caused by P5C.

Multiple distributions of similar samples
None in DPT-NL. This sample was circulated in DPT-France in 2021 (sample F) with overall proficiency 78%.

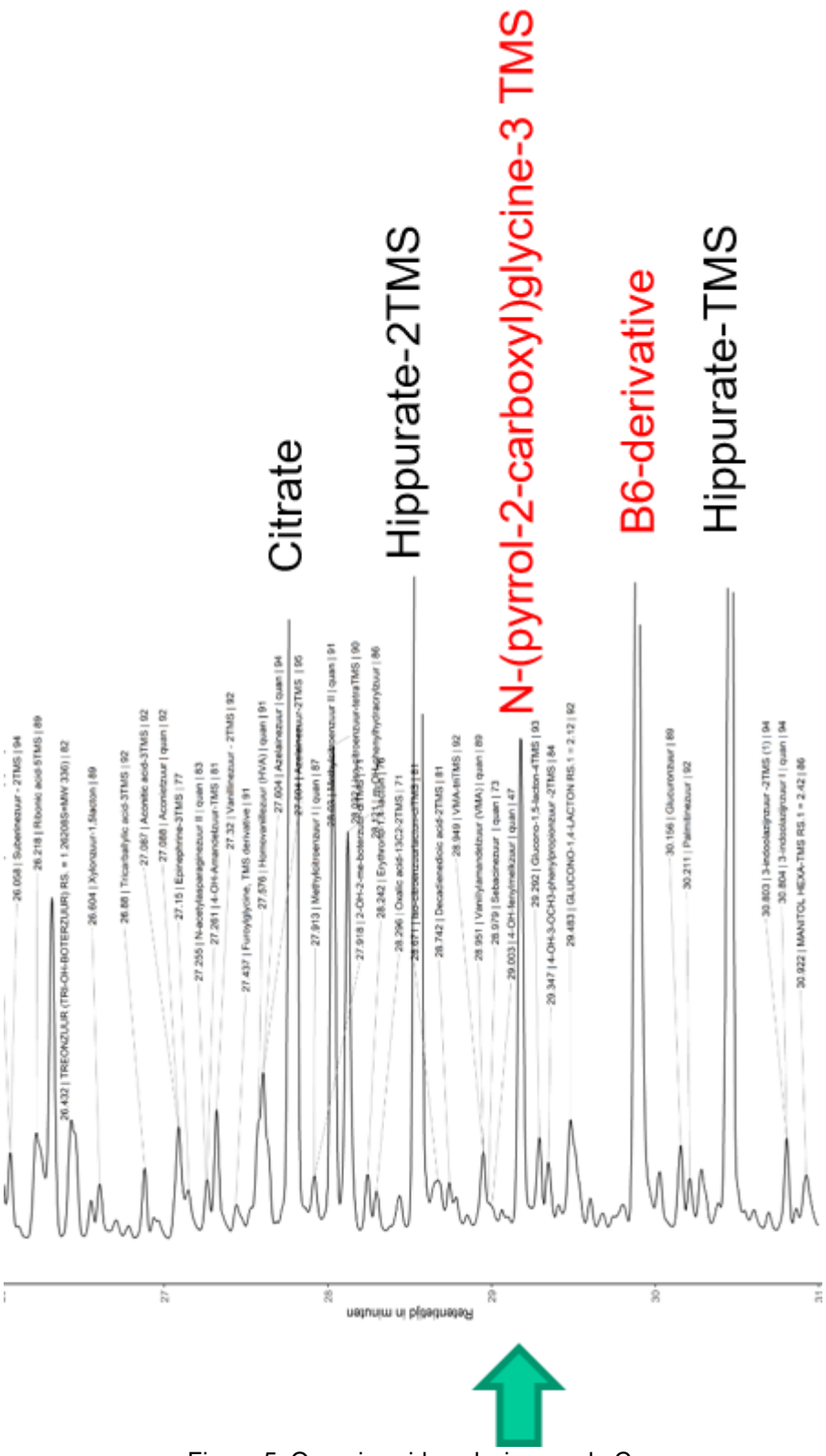

Figure 5. Organic acid analysis sample C.

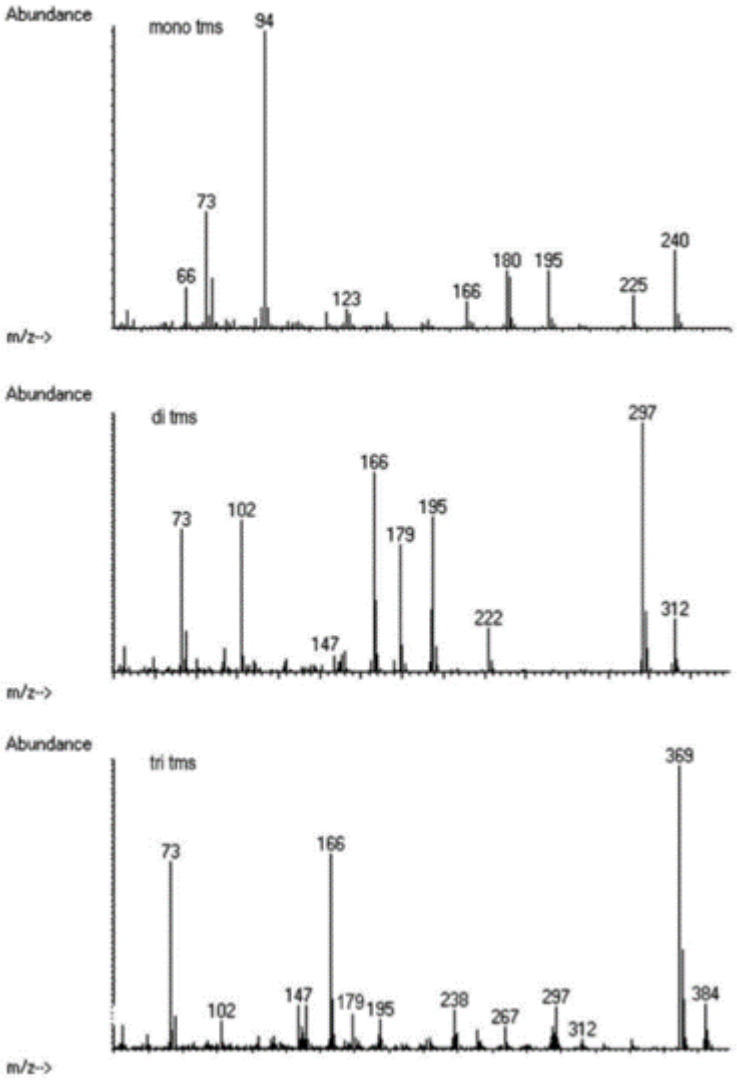

Figure 6. MS spectra of TMS-derivatised pyrrol-2-carboxylglycine (mono-/di-/tr-TMS).

8.5. Patient D – Cerebrotendinous Xanthomatosis (CTX, OMIM 213700)

Patient details provided to participants
Female, investigated at the age of 21 for chronic fatigue, juvenile cataract and slight mental retardation.

Patient details
This female patient was referred to the department of internal medicine at the age of 21 for evaluation of chronic fatigue, juvenile cataract, and slight mental retardation. She was unable to complete an exercise test. The parents were not consanguineous, but both had a low educational level. Based on urine bile alcohol analysis she was diagnosed with CTX
Cataract may result from a number of different IMD. The DD includes RCDP, CTX, Lowe syndrome, defects in galactose metabolism, alfa-mannosidosis, mevalonic aciduria and mitochondrial defects.

Analytical performance
Six out of 17 labs reported elevated C27 bile alcohols (in particular cholestane pentol glucuronide), which was marked 2. Cholestanepentol-glucuronide was 7.7 mmol/mol (ref <0.04) in the SA's laboratory. Also in SCPx deficiency the m/z 627 signal is prominent, but compared to CTX m/z 613 is more prominent. Analytical performance was 35%. A typical urine MS profile of a CTX patient is depicted in Fig. 7.

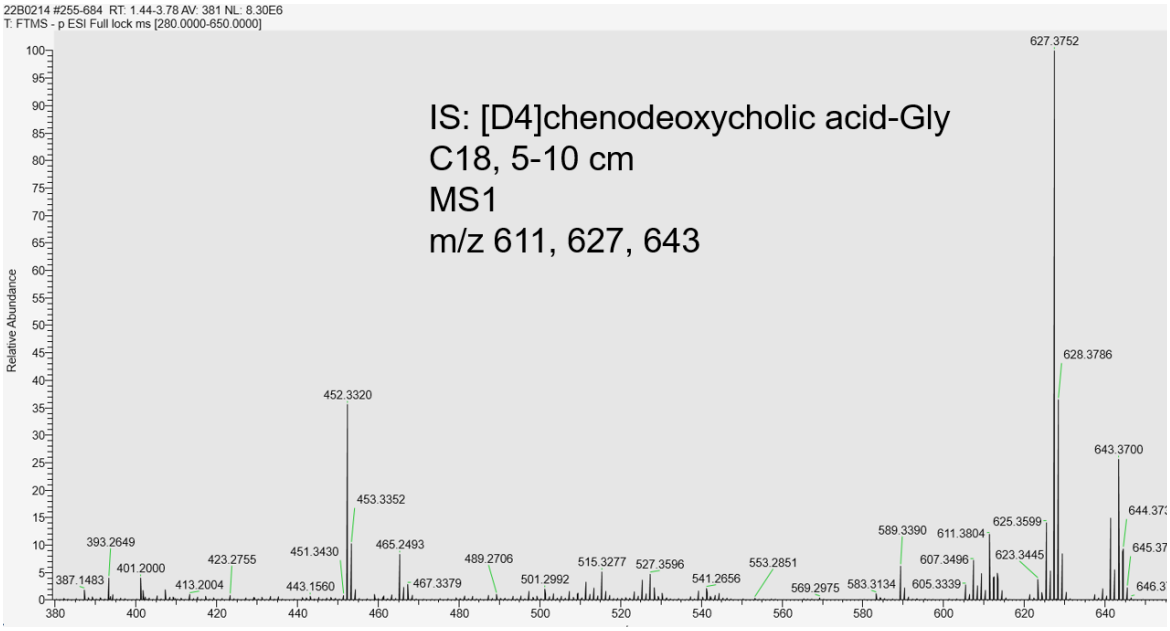

Figure 7. MS1 spectrum of sample D with typical CTX m/z signals.

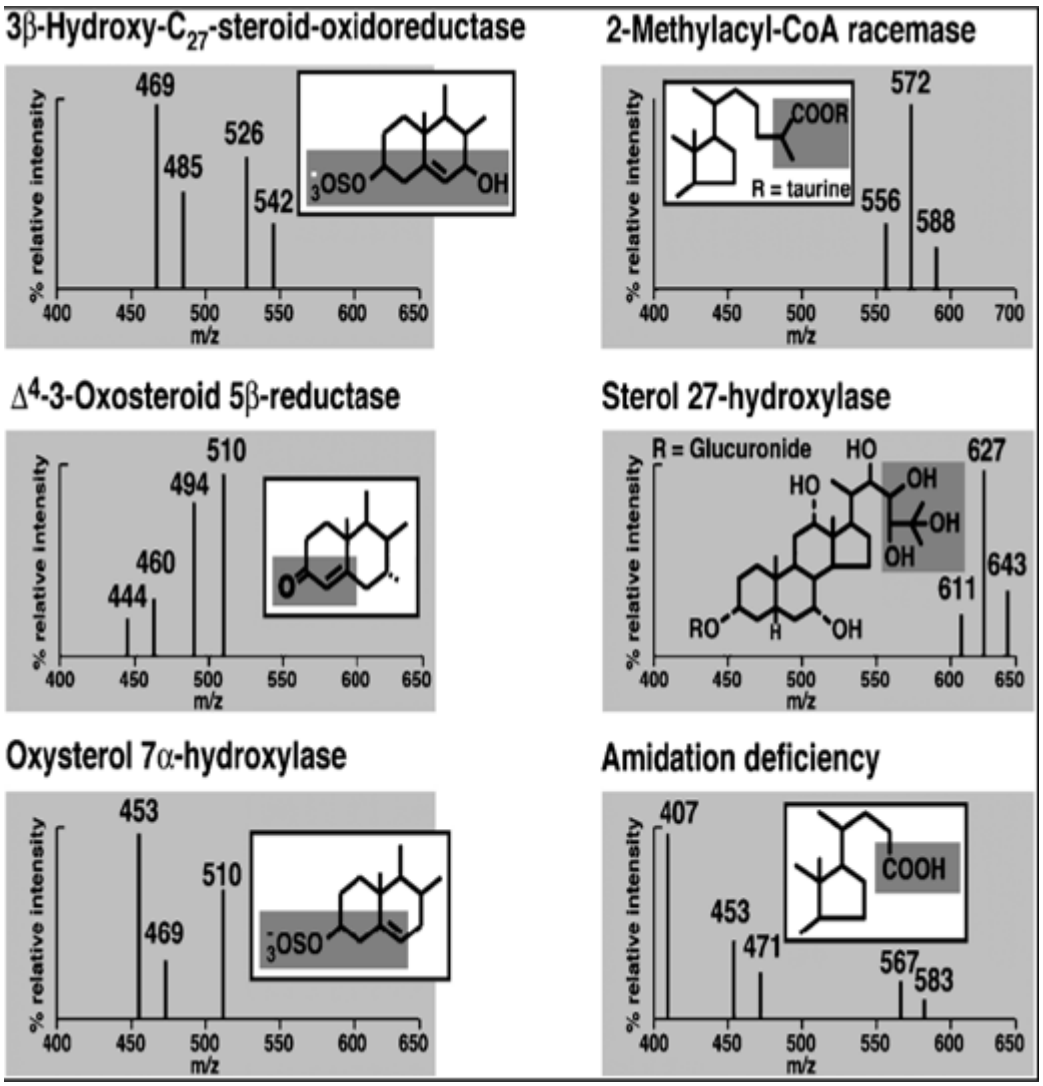

Figure 8. Example spectra of different defects in bile acid biosynthesis (in urine).

Diagnosis / Interpretative proficiency
The six labs that reported elevated C27 bile alcohols all concluded CTX (2 marks). Five participants suggested to investigate CTX based on the clinical symptoms (1 mark). Interpretative performance was 50%.
Bile acids and bile alcohols in urine are diagnostic for defects in bile acid synthesis, such as CTX. Bile acid MS profiles are depicted in Fig. 7 (figure from: Defects in bile acid biosynthesis--diagnosis and treatment. Setchell KD, Heubi JE.2006 J Pediatr Gastroenterol Nutr. 2006 43 Suppl 1:S17-22).
CTX may present with chronic diarrhoea infants and children, while in adults symptoms may include tendon xanthomas, cataract, ataxia and dementia. CTX is a treatable disease (suppletion of chenodeoxycholic acid: Chenofalc/Xenbilox) and should be diagnosed by a Biochemical Genetics laboratory by either urine screening or plasma cholestanol analysis.
No diagnosis/no evidence for an IMD was reported by 6 participants. The following other diagnoses suggested were unlikely (based on urine metabolite findings): MPS IV/homocystinuria? (n=1), Marinesco-Sjogren/mild galactosemia? (n=1) and 3-methylglutaconic aciduria (n=1).
The proficiency of sample D was low and screening of bile acids/bile alcohols is not in the test panel required for participation in DPT schemes. Therefore the SA Board decided this sample would be an educational one (scores of this sample are not included in the total scores and are not considered for performance assessment).

Recommendations
Further investigations reported included determination of cholestanol in plasma and CYP27A1 mutation analysis.

Scoring; sample not scored
Initial scoring criteria:
- Analytical results: abnormal bile alcohols, score 2
- Interpretation of results: CTX, score 2; recommendation to investigate bile alcohols/CTX, score 1
- Critical error: no potential critical errors were identified for this sample

Overall impression
Only six participants have tested bile alcohols, resulting in a low overall proficiency (43%).

Multiple distributions of similar samples
The same sample was circulated in 2013 (sample C) with similar proficiency (36%).

8.6. Patient E – MCAD deficiency (OMIM 201450)

Patient details provided to participants
2-Year old girl presenting with loss of consciousness. The urine sample was collected at age 19 y.

Patient details
Sample E was obtained with the help of the Dutch Patient organisation VKS.

Analytical performance
All but one of the participating labs found at least one of the acylglycines typically found in urine from MCADD patients (2 points). The following metabolites were reported elevated (sorted by decreasing number): hexanoylglycine, phenylpropionylglycine, suberylglycine, 5-OH-hexanoic acid, and suberic acid.
In this sample of an MCADD patient in stable condition, the concentrations of the characteristic metabolites were not high, but yet clearly abnormal. In particular phenylpropionylglycine was easily detected (approx. 90 umol/L). One lab reported abnormal acylcarnitines (e.g. C8, ratio C8/C10), while 3 considered the urine acylcarnitine pattern non-informative.

Diagnosis / Interpretative proficiency
Even though the hexanoyl-/phenylpropionyl-glycine concentrations were not very high, 16/17 participants identified these metabolites and established the correct diagnosis (2 points).
MADD was considered possible by 1 lab, although no other metabolites typical for MADD (such as ethylmalonic acid, 2-hydroxy-glutaric acid, glutaric acid) were abnormal.

Recommendations
Recommendations included plasma free carnitine and acylcarnitine analysis, MCAD activity testing in lymphocytes and ACADM mutation analysis.

Scoring
- Analytical results: any MCADD-specific metabolite (score 2)
- Interpretation of results: MCADD (score 2)
- Critical error: failure to report MCADD. Number of occurrences: 1

Overall impression
This was a straightforward sample, for both analysis and interpretation. Overall proficiency (based on points) was 94%.

Multiple distributions of similar samples
This sample has been previously circulated in 2016 (sample E) with proficiency 96%

8.7. Patient F – Pyroglutamic aciduria due to glutathion synthase deficiency (OMIM 266130)

Patient details provided to participants
A 5-year-old boy with metabolic acidosis since birth, seizures, visual impairment and dental enamel abnormalities. The parents are consanguineous.

Patient details
A homozygous variant of uncertain significance was identified in the GSS gene. In combination with the pyroglutamic aciduria (oxoprolinuria) and the clinical symptoms this indicates glutathion synthase deficiency. Also, a heterozygous, likely pathogenic, variant was identified in the AMELX gene. This result is consistent with the genetic diagnosis of X-linked dominant hypoplastic amelogenesis imperfecta type 1E.
Overproduction of pyroglutamic acid in GS deficiency is due to lack of feedback inhibition of glutamate-cysteine ligase (Fig. 9).

Analytical performance
Grossly elevated pyroglutamic acid (median 9018 mmol/mol) was reported by all participants (analytical proficiency 100%). Some labs mentioned the presence of slightly elevated lactate (median 340 mmol/mol, n=11), succinate (median 180 mmol/mol, n=4), and 3-OH-propionic acid (median 40 mmol/mol, n=3). The cause of these abnormalities is unclear, but may be bacterial metabolism. Acetaminophen, antibiotics (Flucloxacilline) or metabolites from these compounds were not present in the sample.

Diagnosis / Interpretative proficiency
The pyroglutamic aciduria in this sample could be explained by several possible causes. Based on the clinical symptoms provided and the absence of paracetamol and antibiotic (metabolites) in the sample, glutathion synthase deficiency was the most likely diagnosis and this was reported by 16 labs (2 marks). One participant reported pyroglutamic aciduria without mentioning the possibility of GS deficiency (1 mark). Alternative diagnoses mentioned were 5-oxoprolinase deficiency or pyroglutamic aciduria due to secondary causes.
Interpretative proficiency was 97%.

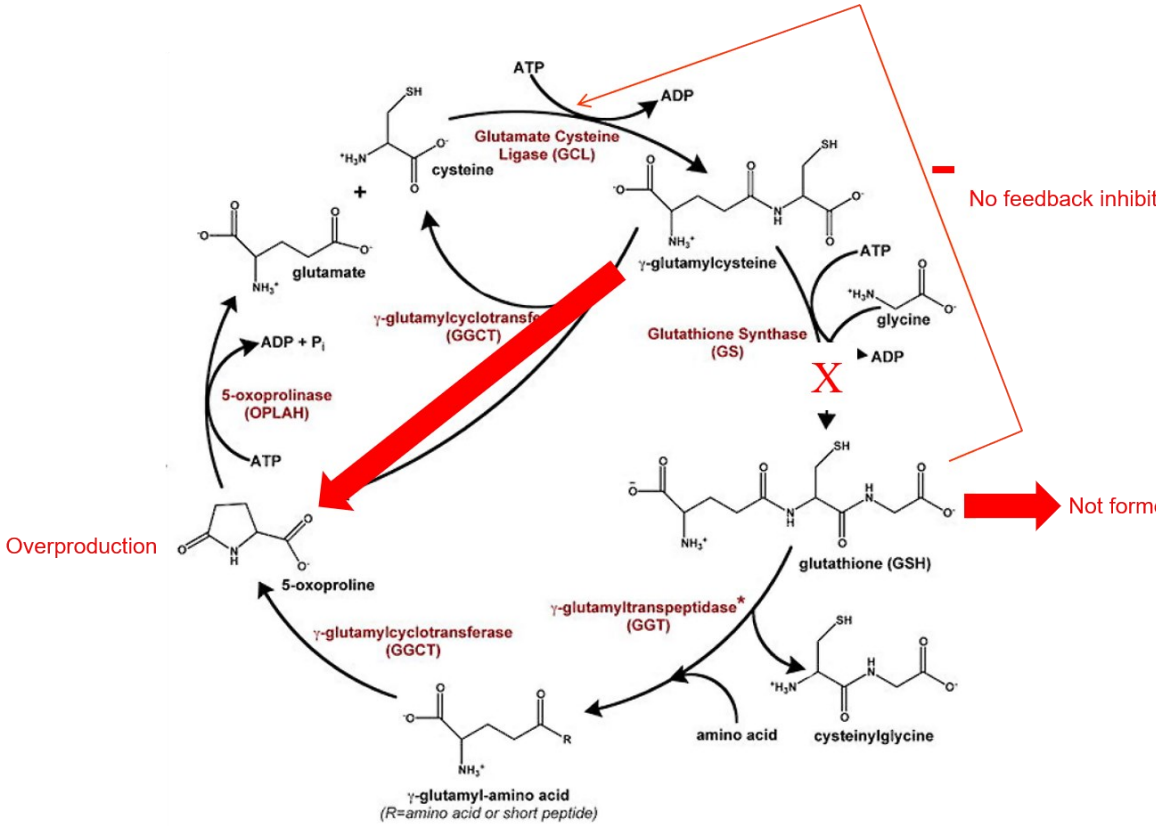

Figure 9. Glutathion cycle showing effects of GS deficiency.

Recommendations
Recommendations made were GSS sequencing, GS activity assay in erythrocytes and glutathion quantification in erythrocytes.

Scoring
- Analytical results: increased pyroglutamic acid (score 2)
- Interpretation of results: glutathione synthase deficiency (score 2), pyroglutamic aciduria without mentioning the possibility of GS deficiency (score 1)
- Critical error: failure to report pyroglutamic aciduria. Number of occurrences: 0

Overall impression
Sample with high overall proficiency: 99%

Multiple distributions of similar samples
In 2021 another oxoprolinuria sample was circulated (2021-C). In this sample the cause was acetaminophen intoxication. The oxoproline excretion in 2021-C was 8688 mmol/mol, which is very similar to the level seen in 2022-F. Apparently the pyroglutamate level cannot discriminate between the 2 different causes.

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.

Detailed scores – Round 1

Lab n°	Patient A Barth syndrome			Patient B I-cell disease			Patient C Hyperprolinemia type II			
	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	1	3	2	2	4	11
3	2	2	4	1	2	3	0	0	0	7
4	2	2	4	2	1	3	1	2	3	10
5	2	2	4	2	2	4	2	2	4	12
6	2	2	4	2	1	3	1	0	1	8
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	1	3	1	2	3	10
10	2	2	4	2	2	4	2	2	4	12
11	2	2	4	1	1	2	2	1	3	9
12	2	2	4	2	1	3	2	2	4	11
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	1	3	1	1	2	9
15	2	2	4	1	1	2	2	2	4	10
16	2	2	4	1	1	2	2	2	4	10
17	2	2	4	2	1	3	1	1	2	9
18	2	2	4	2	1	3	1	0	1	8

Detailed scores – Round 2

Lab n°	Patient D CTX			Patient E MCADD			Patient F GS deficiency			
	A	I	Total	A	I	Total	A	I	Total	Total
1	--	--	--	2	2	4	2	2	4	8
2	--	--	--	--	--	--	--	--	--	0
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	--	--	--	0	0	0	2	1	3	3
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	--	--	--	2	2	4	2	2	4	8
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	3	4	--	--	--	11	55	
3	4	3	0	--	4	4	15	75	CE
4	4	3	3	--	4	4	18	90	
5	4	4	4	--	4	4	20	100	
6	4	3	1	--	4	4	16	80	
7	4	4	4	--	4	4	20	100	
8	4	4	4	--	4	4	20	100	
9	4	3	3	--	0	3	13	65	CE
10	4	4	4	--	4	4	20	100	
11	4	2	3	--	4	4	17	85	
12	4	3	4	--	4	4	19	95	
13	4	4	4	--	4	4	20	100	
14	4	3	2	--	4	4	17	85	
15	4	2	4	--	4	4	18	90	
16	4	2	4	--	4	4	18	90	
17	4	3	2	--	4	4	17	85	
18	4	3	1	--	4	4	16	80	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 70 % of adequate responses)	15	83
Unsatisfactory performers (< 70 % adequate responses and/or critical error)	2	11
Partial and non-submitters	1	6

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-NL-2022-A	Barth syndrome	100	100	100
DPT-NL-2022-B	I-cell disease	89	69	79
DPT-NL-2022-C	Hyperprolinemia type II	78	75	76
DPT-NL-2022-D	CTX	--	--	--
DPT-NL-2022-E	MCADD	94	94	94
DPT-NL-2022-F	GS deficiency	100	97	99

10. Annual meeting of participants

The annual DPT workshop was organised in Freiburg on August 30th 2022 from 9.00 to 10.30. Representatives from approximately half of the participating labs were present.

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

- New **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.
- For validation purposes, **residual batches of EQA materials** (quantitative schemes) are available at (https://www.erndimqa.nl/). Sale will start in January of the year following the scheme year and will continue as long as stocks last.
- **Training**: SSIEM Academy training courses. A 2 days course will be be organized April 24 and 25, 2023 in Manchester. The program includes: organic acidemias, fatty acid oxidation defects and metabolic cardiomyopathies

- **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 2023

Sample distribution	February 8, 2023
Start of analysis of Survey 2023/1 (website open)	March 13, 2023
Survey 2023/1 - Results submission deadline	April 3, 2023
Survey 2023/1 – Interim report available	April/May 2023
Start of analysis of Survey 2023/2 (website open)	June 5, 2023
Survey 2023/2 – Results submission deadline	June 26, 2023
Survey 2023/2 – Interim report available	July/August 2023
Annual meeting of participants	To be announced
Annual Report 2023	December 2023/January 2024

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.

Date of report, January 17, 2023

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

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

END