ERNDIM Administration Office
ERNDIM Administration Office
c/o EMQN CIC, Unit 4, Enterprise House,
Manchester Science Park Pencroft Way
Manchester M15 6SE
United Kingdom.
e-mail: 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: **1)** Alessandro.Salemma@hcuge.ch;
2) Nicola.Braik@hcuge.ch

Published: 26 April 2022[1]

Diagnostic Proficiency Testing

Centre: The Netherlands

Final Report 2021

prepared by
Dr. G.J.G. Ruijter and Dr. W. Onkenhout

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 2021, 19 labs participated in the Proficiency Testing Scheme NL.

1. Geographical distribution of participants

DPT-NL had 19 participants in 2021. The DPT scheme advisors' labs are assigned to one of the other DPT centres and this changes every year. In 2021 the Swiss DPT centre participated in DPT-NL. Due to a mistake in the database this is indicated in the table below as 'undefined country'.

Country	Number of participants	Country	Number of participants
Undefined country	1	Netherlands	8
Australia	2	South Africa	1
Belgium	5	Switzerland	1
Germany	1		

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 CSCQ as scheme organiser (sub-contractor on behalf of ERNDIM), 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 online 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

[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.

Origin of samples: Samples used in 2021 have been provided by:

- Metabolic Center Heidelberg, Germany
- UMCU, Utrecht
- Lady Ridgeway Hospital, Colombo
- Erasmus MC, Rotterdam
- DPT-NL sample repository at Erasmus MC, Rotterdam
- One sample was obtained with help of VKS, the Dutch patient organization

Patient A: Alfa-mannosidosis. Common sample provided by DPT NL
Patient B: Hypophosphatasia
Patient C: Pyroglutamic aciduria
Patient D: MELAS
Patient E: Citrullinemia type 1
Patient F: SSADH 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 9, 2021: shipment of samples
- March 8, 2021: start analysis of samples of the first survey
- March 29, 2021: deadline for result submission (Survey 1)
- May 18, 2021: interim report with preliminary scores of Survey 1 published
- June 7, 2021: start analysis of samples of the second survey
- June 28, 2021: deadline for result submission (Survey 2)
- August 4, 2021: interim report with preliminary scores of Survey 2 published
- September 8, 2021: online DPT workshop
- January 7, 2022: annual report with final scoring published

5. Results

Results were submitted by 19 participants in survey 2021-1, and by 18 participants in survey 2021-2. One participant submitted results late for survey 1. All other participants submitted results for both surveys in time.

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

6. Website 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. 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 as well as a second assessor from another DPT scheme who changes every year. The results of DPT NL 2021 have been scored additionally by Dr Christine Vianey-Saban, from DPT France. At the SAB meeting, November 25-26, 2021, 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. Potential critical errors are discussed and determined by the SAB. Details on critical errors in the 2021 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. One performance support letter will be sent by the Scheme Advisor for DPT-NL 2021. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

A total score of at least 15 points out of the maximum of 24 (62%) and absence of critical errors must be achieved for satisfactory performance in 2021 (please see item 11 for a change in the required minimum score for satisfactory performance in 2022).

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.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was correct in most samples for all labs. Three clearly incorrect values were noticeable in samples B, C and F, but no systematic errors were present. CV's were 4-6% for samples A, B, D and E. Higher CV's were seen in samples C and F due to outliers.

Sample	Median creatinine (mmol/L)	SD (mmol/L)	CV (%)	n
A	3.34	0.20	5.9	19
B	9.10	0.58	6.4	18
C	1.08	0.18	16.7	18
D	4.03	0.15	3.7	18
E	2.15	0.10	4.7	18
F	17.9	1.97	11.0	17

8.2. Patient A – Alfa-mannosidosis (OMIM 248500)

Patient details provided to participants
A 36 year old male with craniosynostosis, dysmorphic facial features, retardation and deafness.

Patient details
The diagnosis was confirmed by enzyme testing in leukocytes (0 mU/mg), plasma (0 mU/mg) and DBS (4.7 pmol/punch; 5% residual activity). No information was available on age of diagnosis, additional clinical information or mutation testing.

Sample A was the common sample distributed to participants of all 5 DPT centers and was discussed during the online ERNDIM participant meeting, October 21, 2021 by dr George Ruijter. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Analytical performance
All participants have investigated oligosaccharides, 13 by TLC and 4 by MS. Two participants obtained MS oligosaccharide results from a partner laboratory. Abnormal oligosaccharide screening results were reported by 18 participants. One participant reported a normal TLC oligosaccharide pattern.
GAG screening was reported abnormal by 11 labs and normal by 6. Further investigation of GAG by electrophoresis/TLC/MS was performed by 11 participants, with 9 finding normal results. GAG apparently are slightly abnormal, which may be explained as secondary to lysosomal dysfunction.
Seven labs reported elevated creatine. Most of these labs interpreted this result as probably secondary to diet or muscle damage.
Analytical proficiency was 95%

Diagnosis / Interpretative proficiency
Alfa-mannosidosis was concluded by 18 participants. Various 'other possible' diagnoses were mentioned such as FGFR mutations, creatine transporter deficiency, mucolipidosis type 2 or 3, GM1 gangliosidosis and 'other lysosomal storage disorders'. The suggestion of creatine transporter deficiency probably relates to the elevated creatin in this sample.
Interpretative proficiency was 95%.

Recommendations
Many participants recommended alfa-mannosidase enzyme testing in WBC (15) and/or MAN2B1 mutation analysis (18). The possibility of ERT was mentioned as well.

Scoring
- Analytical results: abnormal oligosaccharides: score 2
- Interpretation of results: alfa-mannosidosis: score 2
- Critical error: failure to report abnormal oligosaccharides or failure to perform and recommend oligosaccharide analysis. Number of occurrences: 1

Overall impression
Clinical symptoms were suggestive for a lysosomal storage disorder and the oligosaccharide pattern was clearly abnormal. Overall proficiency was 95%.

Multiple distributions of similar samples
In 2018 and 2012 a different alfa-mannosidosis sample was circulated. Proficiencies were 92% in 2018 and 86% in 2012.

8.3. Patient B – Hypophosphatasia due to TNSALP deficiency (OMIM 241510/146300)

Patient details provided to participants
At age 4 this girl presented with delayed motor development and premature loss of teeth. The urine sample was collected at age 18 years.

Patient details
This sample of a hypophosphatasia patient was obtained via the Dutch patient society VKS. The patient was diagnosed at the age of 4 y.
The tissue-non-specific alkaline phosphatase (TNSALP), encoded by the ALPL gene, is one of 4 known alkaline phosphatases and is expressed in liver, kidney, bone and nervous tissues. TNSALP has a role in bone and tooth deposition, presumably by producing inorganic phosphate from various substrates, such as pyrophosphate. It is also required for vitamin B6 metabolism (e.g. conversion of pyridoxal-P to pyridoxal) and dephosphorylation of phosphoethanolamine (PEA).

Analytical performance
Elevated phosphoethanolamine (PEA) was reported by 18 participants. Most participants (17) concluded an elevated/grossly elevated level, one a borderline result. From the quantitative results reported, 12 were around 50 mmol/mol, while one lab reported 6 mmol/mol and one 514 mmol/mol. These latter two values are clearly outliers and might be due to analysis by specific methods or could be clerical errors. During the DPT workshop it was mentioned that PEA values in EDTA plasma are higher than in heparin plasma, since TNSALP activity is dependent on zinc and magnesium.
Phosphoethanolamine is a relatively acidic 'amino acid' and elutes early (between taurine and urea) in Biochrom systems. No other abnormalities were relevant in this sample.
Analytical proficiency was 95%.

Diagnosis / Interpretative proficiency
All participants that identified elevated phosphoethanolamine (18) came to the correct conclusion: hypophosphatasia. The following specific diagnoses were given: hypophosphatasia (n=11), hypophosphatasia childhood (n=4), hypophosphatasia infantile (n=1), (odonto)hypophosphatasia (n=1) and hypophosphatasia intermediate (infantile/adult) (n=1). Hypophosphatasia phenotypes mentioned in literature include: (1) perinatal (lethal), (2) infantile, (3) childhood, (4) adult (heterozygote?), and (5) odontohypophosphatasia. Given the clinical presentation of the patient the childhood/adult phenotype is most likely.
One participant concluded a homocysteine remethylation disorder as a possible diagnosis, presumably based on low homocysteine and methionine values.
Other possible diagnoses reported were: other metabolic bone disease, a disorder of purine/pyrimidine metabolism and folate deficiency (the latter two by the lab that concluded a homocysteine remethylation disorder as a primary diagnosis).
Interpretative proficiency was 97%.

Recommendations
The following recommendations were suggested: alkaline phosphatase activity in serum/plasma, vitamin B6 metabolites (e.g. pyridoxal-phosphate) in serum/plasma, PPi in urine, and mutation analysis of the ALPL gene. With respect to alkaline phosphatase activity testing it is noteworthy that not all clinical chemistry labs have a lower activity cut-off value in their reference range and may therefore not detect decreased activity.
A few labs suggested X-ray/bone scan to establish bone density. Some mentioned the availability of ERT (Asfotase, Strensiq).

Scoring
- Analytical results: elevated phosphoethanolamine: score 2
- Interpretation of results: hypophosphatasia: score 2
- Critical error: failure to report elevated phosphoethanolamine and to mention hypophosphatasia as a possible diagnosis. Number of occurrences: 0

Overall impression
Clearly abnormal sample with grossly elevated phosphoethanolamine. Overall proficiency (based on points) 96%.

Multiple distributions of similar samples
This sample was also circulated in 2014 (proficiency 89%).

8.4. Patient C – Pyroglutamic aciduria due to acetaminophen intoxication.

Patient details provided to participants
Infant treated for gastrointestinal infection presenting with severe metabolic acidosis.

Patient details
This 1 year-old boy was referred for a severe metabolic acidosis. Lactate was 4 mmol/L. Normalisation of organic acids after cessation paracetamol and parenteral feeding excluded a genetic cause of the pyroglutamic aciduria.

Analytical performance
Grossly elevated pyroglutamic acid was reported by all participants. Many participants commented on abnormal excretion of a number of other organic acids (see Fig. 1), including lactate (n=13, median 682 mmol/mol), 4-OH-phenyllactic acid (n=6, median 115 mmol/mol), 2-OH-isovaleric acid (n=4, median 73 mmol/mol), 3-OH-isovalerate (n=4), 3-OH-isobutyrate (n=3), methylmalonic acid (n=3), 3-OH-butyrate (n=2), 2-OH-butyrate (n=2), hydantoin-5-propionate (n=2) and 3-OH-propionate (n=2).
Also a (mild) hyperaminoaciduria was mentioned frequently. Accumulation of formiminoglutamate (FIGLU) was mentioned by two participants.
Twelve labs noted the presence of (metabolites of) acetaminophen/paracetamol and/or antibiotics.
Analytical proficiency was 100%.

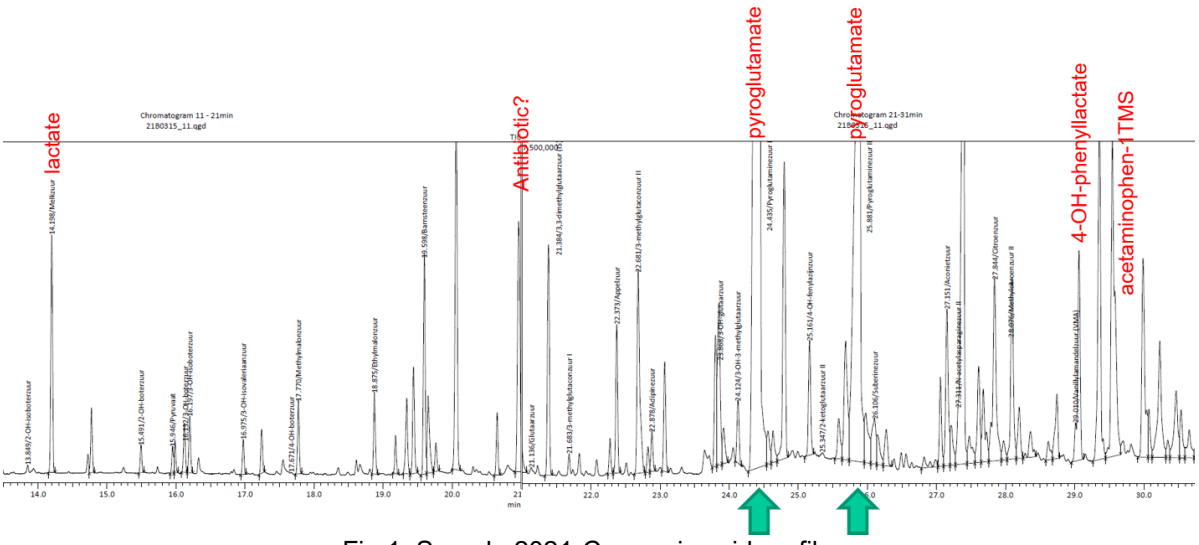

Fig.1. Sample 2021-C organic acid profile.

Diagnosis / Interpretative proficiency
The correct diagnosis in this sample was pyroglutamic aciduria with several possible causes, including glutathion synthase deficiency, oxoprolinase deficiency, or intoxication by medication (paracetamol and/or antibiotics). These 3 possiblities were mentioned by 17 participants either under ‘most likely’ or ‘other possible’ diagnoses, while 2 labs reported only the genetic causes. Five laboratories mentioned that the pyroglutamic aciduria could also be secondary to other IEM. This is formally correct, but unlikely with the clinical description provided with the sample and the observations of the medication metabolites.
Interpretative proficiency was 95%.

The pattern of increases of various organic acids and the mild hyperaminoaciduria may be explained by mitochondrial stress. Elevated FIGLU and hydantoin-5-propionate, both metabolites of histidine degradation, was thought to be secondary to the decompensation or folate deficiency.
Proper functioning of the glutathione cycle may be disturbed by acetaminophen use (Fig. 2). The exact mechanism is unknown. Roles of the GSH cycle include detoxification and amino transport into cells. The GSH-acetaminophen conjugate is removed by filtration in the kidney, but also degradation may occur via the glutathione cycle and lead to pyroglutamic acid (5-oxoproline) overproduction. During the DPT workshop it was mentioned that pyroglutamic aciduria due to paracetamol use is frequently seen in patients in ICU, mostly females. Risk factors include renal failure and flucloxacillin use.

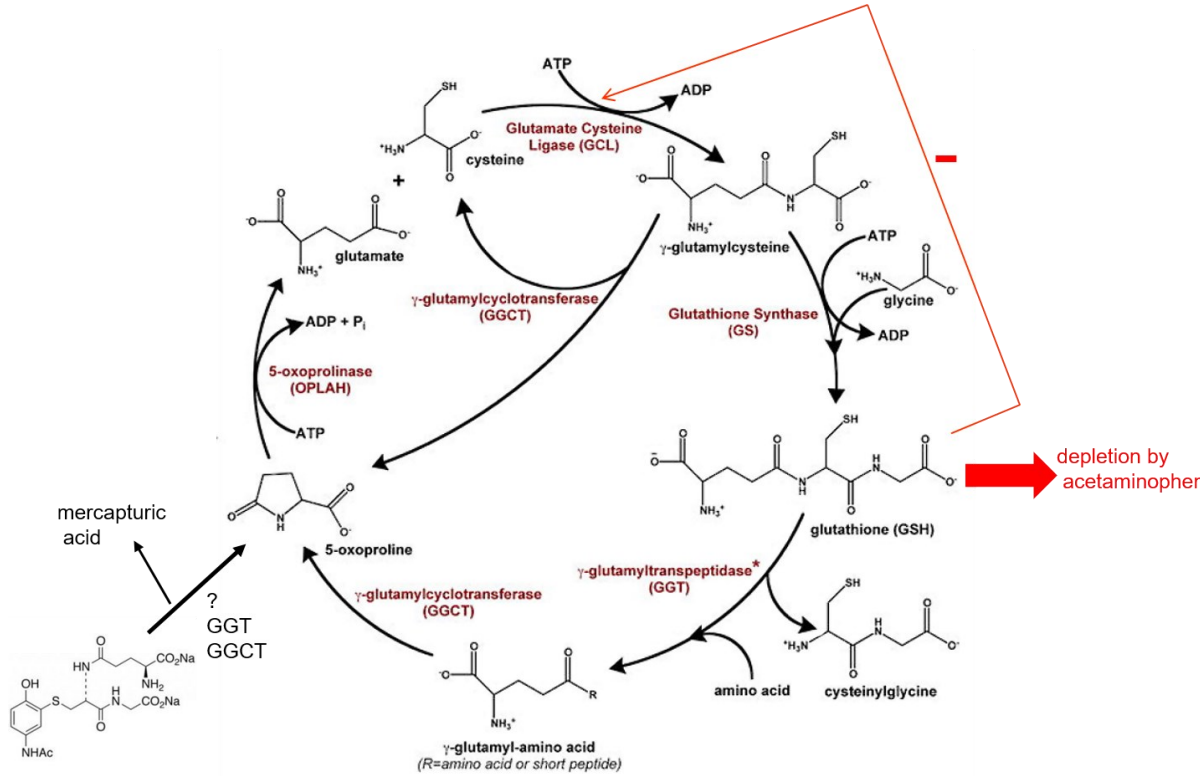

Fig. 2. The glutathion cycle and possible interaction with acetaminophen. The acetaminophen-GSH conjugate may be metabolised via 5-oxoproline (pyroglutamic acid).

Recommendations
Many participants suggested to stop paracetamol and re-analyse organic acids in a stable clinical condition in order to evaluate pyroglutamic acid excretion. Other recommendations made were GSS sequencing and GS activity assay in erythrocytes.
Seven participants suggested treatment by N-acetyl-cysteine. One lab explicitly stated that NAc-cys treatment is not recommended when the primary cause of the pyroglutamic aciduria is not known, because of the possible toxic cysteine accumulation in glutathione synthetase deficiency.

Scoring
- Analytical results: elevated pyroglutamic acid: score 2
- Interpretation of results: pyroglutamic aciduria due to genetic causes or medication: score 2. GS deficiency with secondary causes not mentioned: score 1
- Critical error: failure to report elevated pyroglutamate. Number of occurrences: 0

Overall impression
Overall proficiency was high: 97%

Multiple distributions of similar samples
None

8.5. Patient D – MELAS due to m.3291T>C in MTTL1 (tRNA LEU) mutation (OMIM 590050)

Patient details provided to participants
Girl, aged 8, referred for hypotonia and myopathy.

Patient details
Diagnosis was made by mtDNA sequencing. Blood lactate values have been always elevated, usually above 3 mmol/l. Complex I and IV activities in fibroblasts and muscle tissue were decreased. Following diagnosis she was treated symptomatically and has deceased at age 19.

Analytical performance
Analytical performance with respect to organic acid analysis was excellent. All participants reported grossly elevated lactate (median value 3181 mmol/mol) and most labs mentioned elevations of 3-OH-butyrate (median 980 mmol/mol) and various mitochondrial markers, such as 3-methylglutaconic acid, 3-OH-isobutyrate, fumarate, malate, succinate, aconitate and alanine (see also Fig. 3). Elevated 2,3-di-OH-2-methylbutyrate was reported by 8 labs. Three labs reported a quantitative value for this metabolite, median 17 mmol/mol. Six participants reported elevations of various 3-OH-dicarboxylic acids.
Other metabolites with increased levels and reported by more than one lab were: creatine, carnitine (suppletion), taurine and saccharopine. One participant reported elevated glucose tetrasaccharide, but two others stated that the level of this glycogen catabolite was normal.
Analytical performance was 89%.

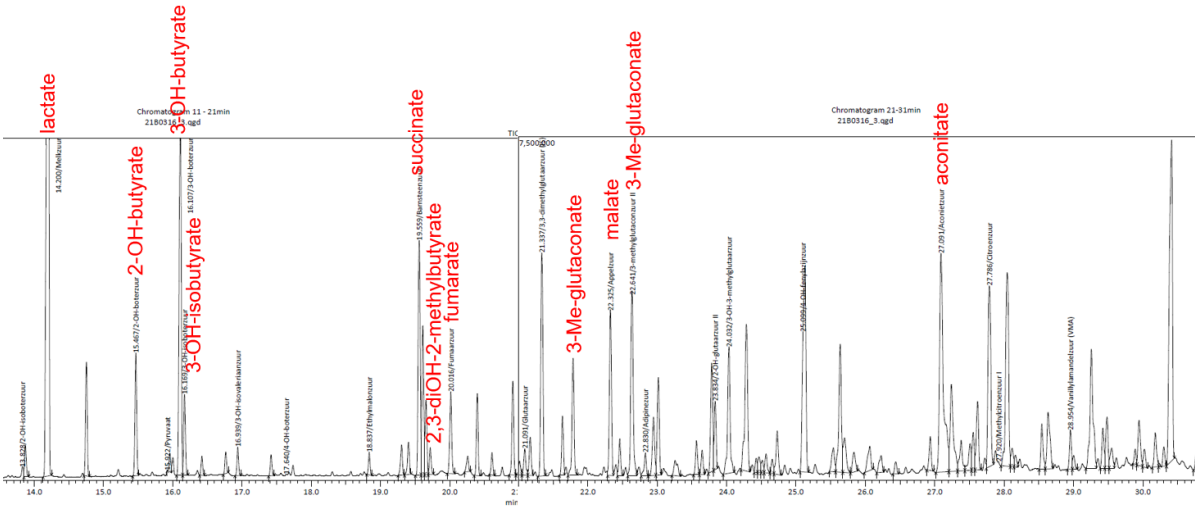

Fig. 3. Sample 2021-D organic acid profile.

Diagnosis / Interpretative proficiency
This survey had 18 participants and 14 reported mitochondrial/oxphos disease as the 'most likely' or 'other possible' diagnosis. Defects in valine catabolism, HIBCH (n=9) and ECHS1 (n=8), were mentioned frequently as possible diagnoses, based on elevations of 3-OH-isobutyric acid and/or 2,3-di-OH-2-methylbutyric acid. The latter is a marker for ECHS1 deficiency, but apparently may be slightly elevated in other mitochondrial disorders and ketosis. In a publication by Peters et al (2015) Mol Gen Metab 115:168-173, reference values for infants are 0-25 mmol/mol, while ECHS1 and HIBCH patients had values in the range of 566-2500. Reference and control values for older children and adults are undecided. Only one participant concluded ECHS1 deficiency without also mentioning the possibility of another (unspecified) mitochondrial disorder.
Some other diagnoses were reported by single participants. LCHAD, GSD 3, mevalonic aciduria and OCTN2 deficiency. Elevations of 3-OH-dicarboxylic acids are most likely aspecific in this case. Glucose tetrasaccharide and mevalonic acid were not abnormal in this sample. In addition, the patient did not suffer from hypoglycemia, and this makes GSD 3 unlikely. Elevated carnitine was reported by 6 participants and interpreted as possibly due to suppletion by 3 participants.
Interpretative proficiency was 78%.

Recommendations
Recommendations focused on mitochondrial investigations: lactate/pyruvate in blood, plasma amino acids, mitochondrial complex activities in muscle/fibroblasts and mtDNA sequencing. Also WES was frequently suggested. Other suggestions for further investigations were: to repeat organic acid analysis, plasma acylcarnitines, ECHS1 and HIBCH mutation analysis, HIBCH activity testing, and analysis of metacrylyl metabolites in urine (e.g. S-(2-carboxypropyl)cysteine).

Scoring
- Analytical results: elevated lactate: 1 point, elevated level of at least 1 other mitochondrial marker: 1 point
- Interpretation of results: mitochondrial disease as first or alternative diagnosis: score 2, specific diagnosis (based on elevated mitochondrial markers) without mentioning mitochondrial disease: score 1
- Critical error: no critical errors were identified for this sample

Overall impression
The metabolic abnormalities in this sample were clear and detected by all participants (high analytical proficiency). Interpretation appeared to be more challenging. Overall proficiency was 83%.

Multiple distributions of similar samples
None

8.6. Patient E – Citrullinemia type 1 (ASS deficiency, OMIM 215700).

Patient details provided to participants
18-Month old boy presenting with an episode of drowsiness following a mild viral infection.

Patient details
After presentation at age 1.5 y the boy was in a state of drowsiness for one day and then recovered. Routine liver and kidney functions were normal. Blood ammonia was not measured, but metabolic investigations led to the diagnosis ASS deficiency. He has no protein aversion and is not treated with nitrogen-scavengers. Citrullinemia was confirmed by 2 mutations in ASS1.

Analytical performance
All 18 participants of survey 2021-2 reported grossly elevated citrulline in this sample (median value 4603 mmol/mol) and 17 mentioned elevated orotic acid (median 34-43 mmol/mol, depending on analysis method). These were the 2 metabolites used for evaluation of analytical proficiency.
A cyclic derivative of citrulline, which is formed spontaneously by intramolecular amidation, was reported by one participant. An organic acid chromatogram and the mass spectrum of this cyclic derivative of citrulline are shown in Fig. 4 and 5.
Elevated homocitrulline was reported as well, with median value 70 mmol/mol. Glutamine and arginine were not abnormal in this sample.
In addition to orotic acid, a number of other pyrimidine metabolites with abnormal high concentration were reported: uracil, uridine, dihydrouracil and 3-ureidopropionate (= n-carbamyl-beta-alanine).
Analytical proficiency was 97%.

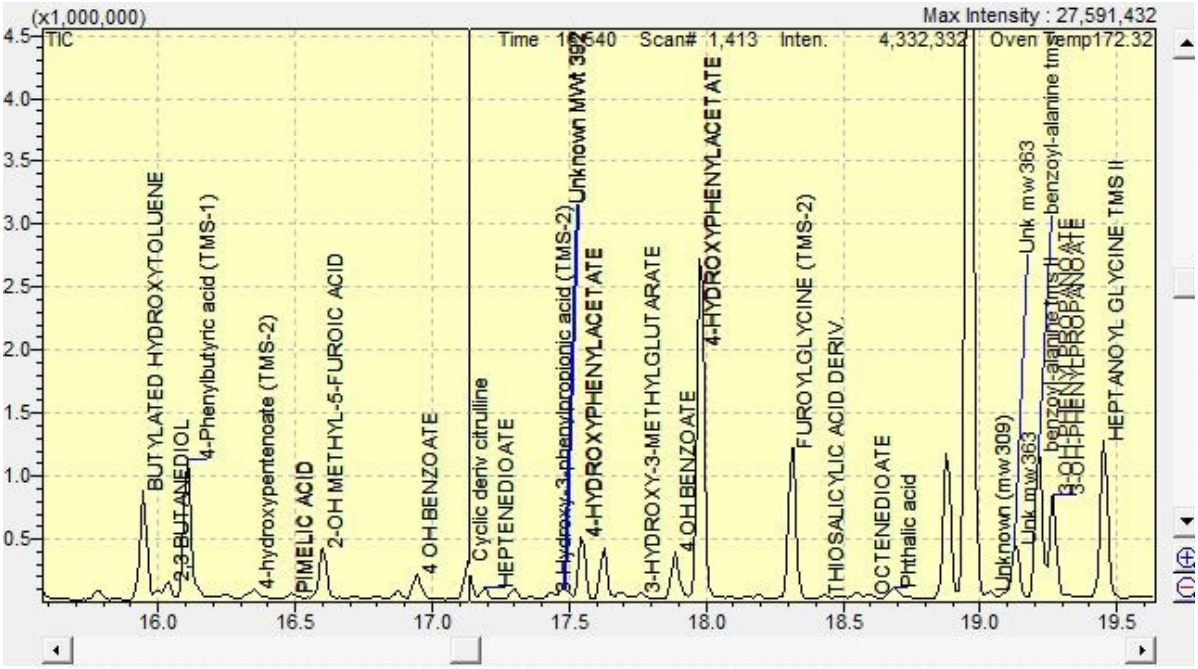

Fig. 4. Sample 2021-E organic acid profile showing cyclic derivative of citrulline (figure provided by dr Joanne Croft, Sheffield).

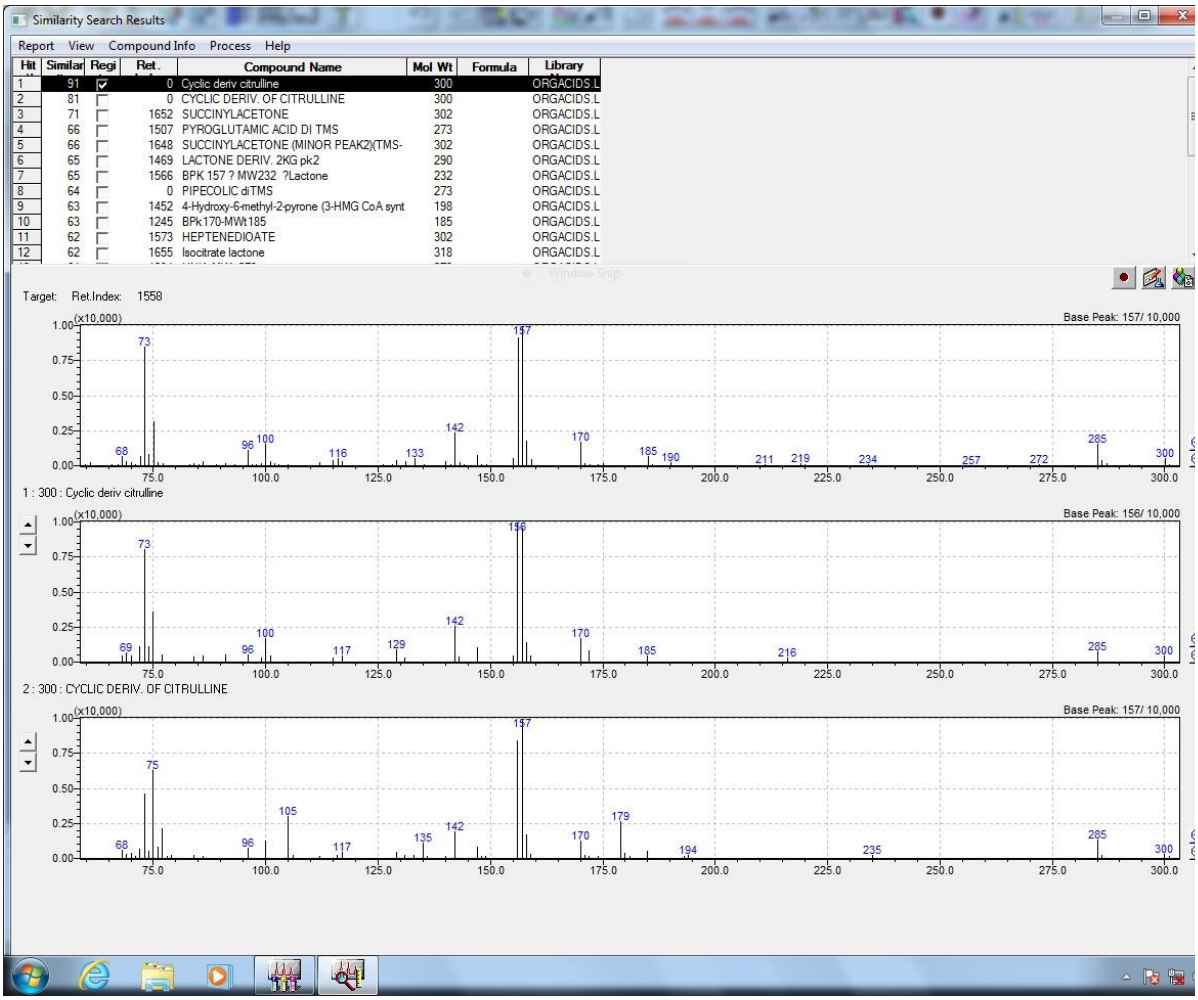

Fig. 5. Image showing mass spectrum of the cyclic derivative of citrulline (figure provided by dr Joanne Croft, Sheffield).

Diagnosis / Interpretative proficiency
All labs concluded citrulinemia as the diagnosis; interpretative proficiency was 100%.
Four participants mentioned under 'other possible' diagnosis the possibility of another urea cycle defect, such as OTC deficiency. This conclusion apparently assumes massive citrulline suppletion, since it would otherwise be unlikely to observe such a high citrulline level.
A number of participants commented on the possibility of citrullinemia type 2: Citrin (SLC25A13) deficiency. Four stated 'Citrin deficiency possible', one 'Citrin deficiency most likely', one 'Citrin deficiency less likely' and one 'Citrin deficiency unlikely'. The latter statement was substantiated by two observations: (1) 'galactose, galactitol are normal' and (2) 'pyrimidine de novo synthesis is not increased in Citrin deficiency'.

Recommendations
Monitoring of blood ammonia and plasma amino acids were mentioned frequently. ASS1 mutation analysis was suggested by 16 participants.

Scoring
- Analytical results: elevated citrulline: 1 point, elevated orotic acid: 1 point
- Interpretation of results: citrullinemia: score 2
- Critical error: failure to report citrullinemia. Number of occurrences: 0

Overall impression
Straightforward sample with high overall proficiency (99%).

Multiple distributions of similar samples
The common sample in 2017 (sample A) was a citrullinemia type 1 sample from a different patient. Overall proficiency was also 99% in 2017.

8.7. Patient F – SSADH deficiency (ALDH5A1 deficiency, OMIM 271980)

Patient details provided to participants
A 14 year old girl with intellectual disability. Seizures had started at age 12.

Patient details
The diagnosis was confirmed by two mutations (nonsense, missense) in the ALDH5A1 gene.

Analytical performance
Elevated 4-OH-butyric acid was reported by 15 participants, while 9 labs mentioned increased 4,5-di-OH-hexanoic acid (lactone, threo-and/or erythro). Three participants stated that the concentration of 4-OH-butyric acid was only mildly elevated. Three participants did not comment on 4-OH-butyric acid or reported a normal level. These labs appeared to use regular GC-MS analysis for organic acid screening. One lab reported increased 3,4-diOH-butyric acid (which is formed from GHB by beta-oxidation). Organic acid analysis is shown in Fig. 6.
Seven labs reported increased glycine.
Analytical proficiency was 83%.

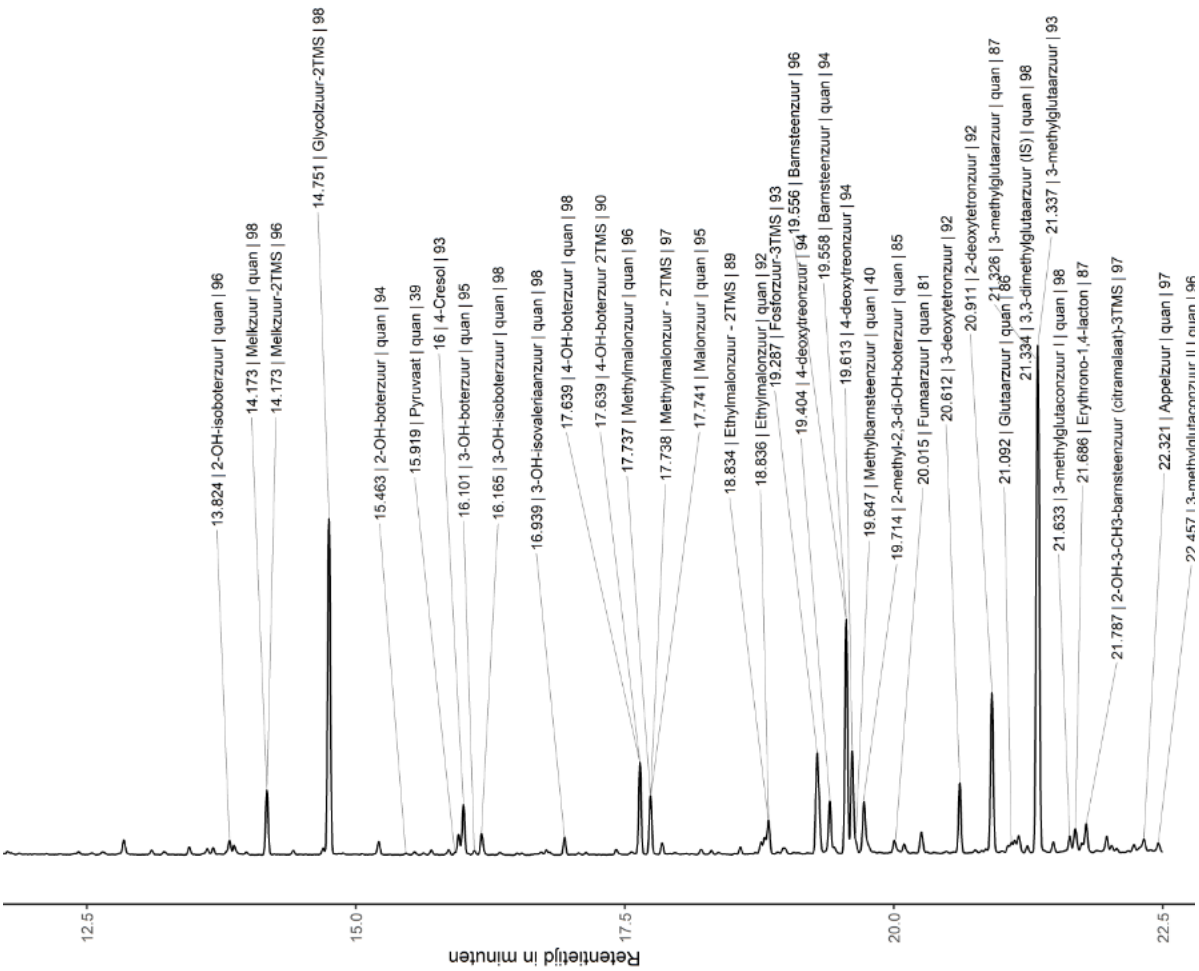

Fig. 6. Sample 2021-F organic acid profile with 4-OH-butyric acid eluting at 17.6 min

Diagnosis / Interpretative proficiency
All labs that detected 4-OH-butyric acid concluded SSADH deficiency (15). One lab still considered SSADH as a possible diagnosis, although 4-OH-butyric acid was not abnormal in their organic acid test. Five participants mentioned that GHB intoxication should be investigated as a possible explanation for the metabolite abnormalities. During the DPT workshop it was mentioned that upon GHB intake no other metabolite abnormalities (e.g. 4,5-diOH-hexanoic acid) would be apparent. Interpretative proficiency was 86%.
The difficulty to diagnose SSADH deficiency in this sample may be explained by two factors. First the level of 4-OH-butyric acid was not very high. This is a known problem in urine sample of older patients. Secondly, 4-OH-butyric acid is a relatively volatile compound and may be lost during sample preparation.
Other diagnoses reported by single participants were MPS IV B and a defect in purine metabolism. GAG and purine metabolites were not clearly abnormal, however. One participant concluded ‘no abnormalities’.

Recommendations
ALDH5A1 mutation analysis was suggested by 15 labs, while 8 recommended to determine SSADH activity in lymphocytes. Two participants suggested to repeat organic acid analysis.

Scoring
- Analytical results: elevated 4-OH-butyric acid: score 2
- Interpretation of results: SSADH deficiency: score 2
- Critical error: no critical errors were identified for this sample

Overall impression
A somewhat challenging sample due to relatively low 4-OH-butyric acid level. Overall proficiency 85%.

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.

Detailed scores – Round 1

Lab n°	Patient A Alfa-mannosidosis			Patient B Hypophosphatasia			Patient C Pyroglutamic 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	2	4	2	2	4	2	2	4	12
8	2	2	4	2	2	4	2	2	4	12
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	2	2	4	2	2	4	12
11	2	2	4	2	2	4	2	2	4	12
12	2	2	4	2	2	4	2	2	4	12
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	2	1	3	11
15	2	2	4	2	2	4	2	2	4	12
16	0	0	0	2	2	4	2	1	3	7
17	2	2	4	2	2	4	2	2	4	12
18	2	2	4	2	2	4	2	2	4	12
19	2	2	4	0	1	1	2	2	4	9
20	--	--	--	--	--	--	--	--	--	0

Detailed scores – Round 2

Lab n°	Patient D MELAS			Patient E Citrullinemia type 1			Patient F SSADH deficiency			
	A	I	Total	A	I	Total	A	I	Total	Total
1	--	--	--	--	--	--	--	--	--	0
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	2	2	4	2	2	4	12
9	1	0	1	2	2	4	2	2	4	9
10	2	2	4	2	2	4	2	2	4	12
11	1	0	1	1	2	3	0	0	0	4
12	2	2	4	2	2	4	0	0	0	8
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	2	2	4	12
15	2	2	4	2	2	4	2	2	4	12
16	2	1	3	2	2	4	2	2	4	11
17	2	2	4	2	2	4	2	2	4	12
18	1	0	1	2	2	4	2	2	4	9
19	1	1	2	2	2	4	0	1	1	7
20	--	--	--	--	--	--	--	--	--	0

Total scores

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)	Critical error
1	4	4	4	--	--	--	12	50	
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	4	4	4	24	100	
8	4	4	4	4	4	4	24	100	
9	4	4	4	1	4	4	21	88	
10	4	4	4	4	4	4	24	100	
11	4	4	4	1	3	0	16	67	
12	4	4	4	4	4	0	20	83	
13	4	4	4	4	4	4	24	100	
14	4	4	3	4	4	4	23	96	
15	4	4	4	4	4	4	24	100	
16	0	4	3	3	4	4	18	75	CE
17	4	4	4	4	4	4	24	100	
18	4	4	4	1	4	4	21	88	
19	4	1	4	2	4	1	16	67	
20	--	--	--	--	--	--	0	0	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	17	90
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	1	5
Partial and non-submitters	1	5

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-NL-2021-A	Alfa-mannosidosis	95	95	95
DPT-NL-2021-B	Hypophosphatasia	95	97	96
DPT-NL-2021-C	Pyroglutamic aciduria	100	95	97
DPT-NL-2021-D	MELAS	89	78	83
DPT-NL-2021-E	Citrullinemia type 1	97	100	99
DPT-NL-2021-F	SSADH deficiency	83	86	85

10. Annual participant meeting

The annual DPT workshop was held online on September 8, 2021. The online nature of the meeting enabled representatives from many participating labs to attend.

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

- **Performance assessment of DPT schemes.** The SAB has decided to increase the minimum total score required for satisfactory performance to 17 points out of the maximum of 24 (71%) starting in the 2022 scheme year. This used to be 15/24 (62%) up to the 2021 scheme year. Re-evaluation of the minimum required score is done regularly by the SAB in order to adapt minimum scores to actual performance in single scheme years (e.g. when a sample is classed educational) and to harmonise the required minimum scores across all interpretive schemes (DPT, QLOU, ACDB, CDG and UMPS).
- **New control materials**. QC samples are available for the following groups of analytes: amino acids, organic acids, purines-pyrimidines, acylcarnitines and special assays. New in 2020 have been the QC samples for pterins. 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 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.
- **New schemes.** The Amino Acid Interpretation scheme will be organized as a pilot scheme still in 2022, but will most probably be a regular scheme in 2023. The SAB is considering to start a new special assays scheme for lipids and sterols. The number of lipids and sterols analysed by IMD

laboratories is growing and it is increasingly difficult to include these in the Special Assays Serum scheme. This new Special Assays Lipid scheme will probably be introduced as a regular scheme from the start. A possible new pilot scheme will be a metabolomics scheme using plasma or serum as a sample type. In the participant surveys we have had requests to start schemes for lysosomal enzymes in DBS and qualitative bile acids in urine. No firm plans are in place to start these as introduction is hampered by sample availability.

- **Training**: Currently, an SSIEM Academy training course is planned to be held in Amsterdam in April. Topics include aminoacidopathies, hyperammonaemia and urea cycle disorders. Information can be found on the SSIEM and ERNDIM websites.
- **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 250 ml of urine from a patient affected with an established inborn error of metabolism, accompanied by a short clinical report. If possible, please collect 1250 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 2022

Sample distribution	February 2, 2022
Start of analysis of Survey 2022/1 (website open)	March 14, 2022
Survey 2022/1 - Results submission deadline	April 4, 2022
Survey 2022/1 – Interim report available	April/May 2022
Start of analysis of Survey 2022/2 (website open)	June 6, 2022
Survey 2022/2 – Results submission deadline	June 28, 2022
Survey 2022/2 – Interim report available	July/August 2022
Annual meeting of participants	August 30, 2022 (Freiburg)
Annual Report 2022	January 2023

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, 2022-01-07
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	26 April 2022	2021 annual report published

END