ERNDIM
Diagnostic Proficiency Testing
DPT Centre: Netherlands

ANNUAL REPORT 2017

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1. Introduction

The ERNDIM Diagnostic Proficiency Testing (DPT) Scheme is the ultimate external quality assessment scheme for biochemical genetics laboratories. The minimal required test panel for participation in any DPT scheme includes creatinine, dip stick, amino acids, organic acids, oligosaccharides and quantitative GAG. DPT-NL additionally requires the analysis of 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 neighbouring laboratories when this is routine clinical practice. It is required to indicate in the report that results were obtained from a cluster lab.

2. Participants

The 2017 scheme had 21 participating laboratories with the allocations listed in Table 1. Twenty participants have submitted results for both surveys. One participant submitted results of the second survey only.

Table 1. Participants in DPT-NL 2017

Country	Number of participants
Australia	3
Belgium	5
Germany	2
India	1
Netherlands	8
South-Africa	1
Switzerland	1

3. Logistics of the scheme

The samples used in the DPT scheme are authentic human urine samples and were selected by the Scientific Advisors of the scheme (Table 2). 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. Two surveys were performed; 2017-1 (samples A, B, C) starting February 20, and 2017-2 (samples D, E, F) starting May 22. 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. Sample dispatch was done February 6, 2017 by DHL.
Reports of the samples were submitted electronically to the website of the Swiss organisation for quality control (CSCQ) (https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php). The time allotted for submitting reports was 3 weeks after opening of the website. Clinical information on the samples was provided through the website.

Table 2. The 2017 DPT NL samples. Samples were provided by dr Heiner (Groningen, The Netherlands), dr Croft (Sheffield, United Kingdom, DPT-UK) and dr Oussoren, dr de Klerk and dr Williams (Rotterdam, The Netherlands).

Sample	Diagnosis
A	Citrullinemia type I
B	D-2-OH-glutaric aciduria type 2
C	DPD deficiency
D	Hartnup disease
E	GM1 gangliosidosis
F	Lysinuric Protein Intolerance

4. Scoring of results

General scoring criteria are depicted in Table 3. Scoring of the 2017 samples was performed according to the criteria summarised in Table 4. In order to achieve harmonised scoring throughout the five DPT schemes, each of the DPT schemes is evaluated by a second scoring officer belonging to one of the partner DPT schemes. The external scores are discussed with the scheme's own scientific advisor(s). For the DPT-NL scheme, the second evaluation was performed by the scientific advisor of the DPT France scheme in 2017 (dr C. Vianey-Saban).

Table 3. General criteria for scoring results.

Item	Criterium	Score
Analytical performance:	Correct results of the appropriate tests	2
	Partially correct or non-standard methods	1
	Unsatisfactory or misleading	0
Interpretative performance:	Good (diagnosis was established) and adequate recommendations were suggested	2
	Helpful but incomplete	1
	Misleading / wrong diagnosis	0
	Total maximal score for each sample	4

Table 4. Specific criteria for scoring results of the 2017 samples.

Sample	Analytical	points	Interpretation	points
A	elevated citrulline elevated orotic acid	1 1	citrullinemia other UCD	2 1
B	elevated 2-OH glutaric acid	2	diagnosis D-2-OH-glutaric aciduria based on enantiomer analysis 2-OH-glutaric aciduria recommendation to perform enantiomer analysis or genetic analyses	2 1 1
C	elevated uracil elevated thymine	1 1	DPD	2
D	elevated (neutral) amino acids	2	Hartnup tubular damage/renal Fanconi	2 1
E	abnormal oligosaccharides	2	GM1 any other oligosaccharidosis or oligosaccharidosis unspecified recommendation to perform oligo analysis	2 1 1
F	elevated lysine elevated uracil or orotic acid	1 1	LPI UCD	2 1

The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. In accordance with a previous decision by the board, participants who failed to achieve satisfactory performance were those who scored less than 15 points out of the maximum of 24 in this year. Starting with the 2014 schemes the concept of 'critical error' is introduced to the assessment of the DPT schemes. 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 by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on November 23, 2017. The critical errors identified in the 2017 scheme are listed in Table 5. Previously, critical errors were not applied in 'normal' samples (i.e. without IEM). This has been changed in 2017. In future surveys a normal sample may be eligible for critical error if (1) a patient is not suspected of an IEM, and (2) the majority of participants do not mention abnormalities leading to diagnosis, and (3) the reported diagnosis could lead to harmful treatment.

Table 5. Critical errors in the 2017 scheme.

Sample	Critical error	No. of occurences
A	Failure to report both citrulline and orotic acid	0
B	Failure to detect 2-OH-glutaric acid	0
C	None	-
D	Failure to report hyperaminoaciduria	0
E	Failure to detect abnormal oligosaccharides or failure to perform oligosaccharide analysis and no recommendation to do so	1
F	None	-

5. Communication of results

The CSCQ scheme evaluation programme was used to generate individual lab reports and these were distributed on April 19th and August 14th 2017. These individual participant reports included the scores obtained. Scores are still preliminary in interim reports, since the 2nd evaluation has not been done.
Discussion of the results took place in Manchester during the ERNDIM participant meeting, November 21, 2017. The meeting, open to participants only, was attended by 17 representatives from 9 of the participating institutes. Two participants have sent notifications of their absence at the DPT meeting. The scientific advisor of the scheme, George Ruijter (Erasmus Medical Centre Rotterdam) chaired the meeting and presented the analytical/diagnostic points of interest. Items discussed during the meeting have been included in the current annual report. The presentation used during the workshop is available from the ERMDIM website (erndim.org > meetings & reports).

ERNDIM provides a single certificate for all its schemes with details of participation and performance.

One Performance Support letter will be send for the 2017 surveys. Two were issued in 2016.

6. Proficiency of the 2017 surveys

Proficiencies (% of maximal achievable points for all labs) of the 2017 samples are summarized in Table 6. Distribution of scores is given in Table 7.
Maximal scores (24 points) were obtained by 11 out of the 20 participating labs, which is considerably more than in 2016 (2 participants with full score). Eighteen labs scored at least 20 p. Samples A and B were rather straightforward, while sample C, D, E and F were a bit more challenging. Overall performance for all six samples was 92%, which is much better than in previous years. It is tempting to conclude that performance in IEM diagnostics is improving over the years, but it must be noted that overall performance in the DPT scheme may vary with changes in participating labs and with the samples selected by the scientific advisor.

Table 6. Performance on the DPT 2017 samples.

Sample	Diagnosis	No. of reports	Proficiency (%)		
			analytical	interpretation	TOTAL
A	Citrullinemia type I	20	98	100	99
B	D-2-OH-glutaric aciduria type 2	20	100	98	99
C	DPD deficiency	20	93	90	91
D	Hartnup disease	21	98	88	93
E	GM1 gangliosidosis	21	90	86	88
F	Lysinuric Protein Intolerance	21	81	79	80

Table 7. Distribution of final scores in 2017; for each sample the number of participants with score 0/1/2/3/4 points is given. Score 0 due to non-submission of results is not included.

Sample	0 points	1	2	3	4
A	0	0	0	1	19
B	0	0	0	1	19
C	1	1	0	0	18
D	0	1	1	1	18
E	1	1	0	3	16
F	2	2	1	1	15

7. Results of individual samples and evaluation of reported results

Sample 2017-A: Citrullinemia type 1 (Argininosuccinate synthase deficiency, OMIM 215700).

Clinical description: 3 day-old baby who presented to his local A&E. The clinical details at the time were 'febrile infection'.

Sample A was the common sample distributed to participants of all 5 DPT centers and was discussed during the ERNDIM participant meeting in Manchester, November 22, 2017 by dr Croft from Sheffield. The presentation showing results and conclusions on this sample can be viewed on the ERNDIM website (erndim.org).

Sample 2017-B: D-2-OH-glutaric aciduria type 2 (Isocitrate dehydrogenase 2 mutation; OMIM 613657).

Clinical description: An 11-month old boy presenting with muscle weakness and mild psychomotor retardation.
D-2-OH-glutaric aciduria type 2 is caused by a germline mutation in IDH2. The diagnosis was confirmed by mutation R140G in IDH2 in this patient.

Analytical.
All participants reported elevated 2-hydroxy-glutaric acid and 4 participants specifically reported elevation of D-2HGA (1031 mmol/mol; 613 mmol/mol; 95% D-isomer; 90% D-isomer). Analytical proficiency was 100%. The following concentrations were reported:

	median	range	
Creatinine	1.3	1.2-1.5	mmol/L
2-OH-glutaric acid	1897 (n=13)	212-4649	mmol/mol
2-keto-glutaric acid	0 (n=5)	0-3	mmol/mol

Interpretation.
All participants concluded that 2-hydroxy-glutaric aciduria was the most likely diagnosis. The 4 labs that performed enantiomer analysis were able to conclude that this sample was obtained from a D-2HGA patient, while of the remaining 16 participants, 15 recommended enantiomer analysis. Interpretative proficiency was 98%.
Other recommendations for further investigations included mutation analysis of 2-4 genes possibly causing the disease:

Excretion pattern	gene	inheritance
D-2-OH-glutaric aciduria	D2HGDH	AR
	IDH2	AD, de novo
L-2-OH-glutaric aciduria	L2HGDH	AR
D/L-2-OH-glutaric aciduria	SLC25A1	AR

During the DPT meeting it was concluded that the level of (total) 2-HGA determined in initial organic acid screening is not informative for the underlying genetic defect causing the 2-OH-glutaric aciduria. Only in case of a relatively moderate excretion level, this may be suggestive for combined D/L-2-OH-glutaric aciduria due to SLC25A1 deficiency. To some extent clinical symptoms may guide the diagnosis (cardiomyopathy: D2HGDH, leukoencephalopathy: L2HGDH).
Seven participants reported elevated lysine in the urine sample. In L-2-OH-glutaric aciduria it has been suggested that high CSF lysine levels are related to a deficiency of 2-ketoglutaric acid (2KG). 2KG is required for lysine catabolism and is converted to 2HGA in 2-OH-glutaric aciduria. A similar

mechanism may operate in D-2-OH-glutaric aciduria and this may explain low 2KG level and possibly high lysine in sample DPT 2017-B. It must be noted that 2KG might also be degraded in the urine sample due to storage or sample processing.

Overall proficiency (based on points) 99%
Diagnosis of 2-OH-glutaric aciduria in this sample was straightforward.

Sample 2017-C: Dihydropyrimidine dehydrogenase deficiency (DPD deficiency; OMIM 274270).

Clinical description: At the age of 5 y, this girl was investigated for seizures. The urine sample was collected at age 9 y.

DPD enzyme activity (Amsterdam) was deficient with 4% residual activity and 2 mutations were found in the DPYD gene; 1 missense and 1 nonsense, confirming the diagnosis.
The patient is treated with valproic acid.

Analytical.
Labs performing purine and pyrimidine analysis were able to detect increased uracil and thymine (n=18/20). A number of participants reported elevated uracil in organic acid analysis as well. Analytical proficiency was 93%. The following excretions were reported (purine-pyrimidine analysis; mmol/mol):

	median	range	n
Uracil	135	76-218	15
Thymine	27	11-41	15

One participant reported absence of 5-hydroxymethyl-uracil, but one lab found elevated 5-hydroxymethyl-uracil. Six labs stated that dihydro-uracil and dihydro-thymine excretion was normal, while one participant reported elevated levels of these 2 metabolites. Only one participant reported thymidine and deoxyuridine, which were considered normal.
Many labs reported an increased value of glycine (15/20). Four concluded that this was caused by valproic acid use, which is a known cause of elevated glycine.

Interpretation.
All 18 participants that determined purines-pyrimidines mentioned DPD deficiency as the most likely or other possible diagnosis. With elevated uracil and thymine, the differential diagnosis includes dihydropyriminidase (DHP) deficiency and MNGIE. DHP deficiency is less likely due to normal dihydro-uracil and dihydro-thymine excretion. MNGIE is unlikely because of normal thymidine and deoxyuridine levels.
During the DPT meeting it was stated that absence of 5-hydroxymethyl-uracil does not rule out DPD deficiency, but is rather atypical. β-Ureidopropionase (UPB) deficiency was suggested as an alternative diagnosis, but since uracil and thymine are not elevated in UPB deficiency, this diagnosis was considered unlikely. Finally, it was noted that particular anti-HIV medication may result in elevated uracil and thymine.

The following diagnoses were reported:

Diagnosis	Most likely	Other possible	Comment
DPD def	16	2	
DHP def	1	4	dihydroUra, dihydroThy normal
ThyUra-uria	1	-	
NKH	1	-	elevated gly due to valproate/bacterial contamination
AGAT def	1	-	cre (171), gua (38 mmol/mol); both normal
UPB def	-	2	Ura, Thy not elevated in UPB def

A challenging aspect of this sample was the obvious bacterial contamination. Various observations were made that indicated bacterial growth in the sample: nitrite-positive (n=13), lactate elevated (n=9),

serine low/decreased (n=3) and benzoate elevated (n=4). Bacteria might have (partially) caused elevated glycine due to hydrolysis of hippuric acid to benzoate and glycine as well as elevated uracil due to degradation of pseudo-uridine. Therefore, the elevation of thymine was crucial to conclude DPD deficiency in this sample.
Recommendations for further investigations included: DPD enzyme assay (in lymphocytes) and DPYD mutation analysis.

A recommendation that was considered rather essential by many participants was to mention 5-fluorouracil toxicity, not just in the patient, but also in relatives such as parents and sibs.

While the diagnosis was made at age 5 y, a urine sample was available from the patient collected at age 1 day. This sample was not analysed for purines-pyrimidines at the time of sampling. After establishing the diagnosis the early sample was subjected to purine-pyrimidine analysis. Surprisingly, uracil and thymine were normal. It is unknown whether uracil and thymine in this sample were normal at the time the urine was collected. Consensus during the DPT meeting was that uracil and thymine are not unstable compounds and are thought not to be degraded during 5 y of storage at -20°C. Also it was expected that uracil and thymine would be elevated in a DPD patient at age one day leaving this issue unresolved.

Overall proficiency (based on points) 91%
Correct interpretation required pyrimidine analysis.

Sample 2017-D: Hartnup disease (OMIM 234500).

Clinical description: 12 year old male with cerebellar ataxia.

Confirmation of the diagnosis, e.g. by mutation analysis, was not available.

Analytical.
All 21 laboratories noted abnormal amino acids in this sample (Fig. 1). The most frequently reported elevated amino acids were glutamic acid, valine and leucine (each n=15), isoleucine (n=12), phenylalanine (n=10) and alanine (n= 8). Increased neutral amino acids was reported by 16/21 labs. Four described the pattern as generalized aminoaciduria, while one participant interpreted the pattern as fitting with MSUD.

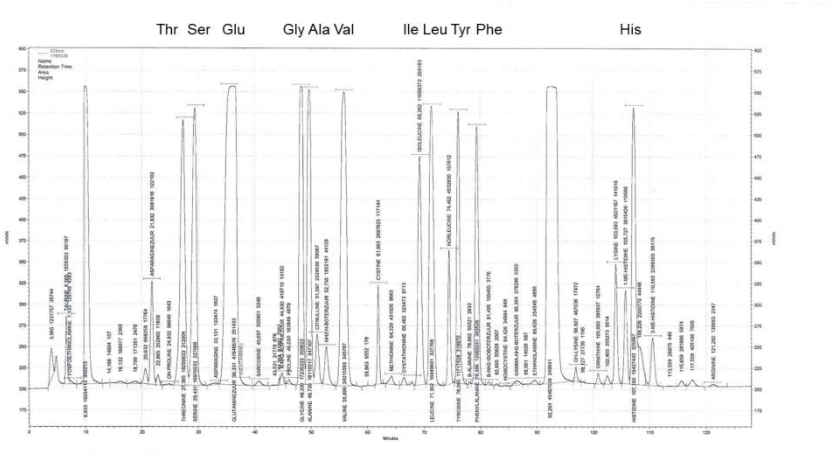

Fig 1. Amino acid analysis of sample DPT-NL 2017-D using Biochrom 30. Strongly elevated amino acids are indicated.

Interpretation.
Eighteen labs correctly diagnosed Hartnup disease. Of the remaining 3 participants who did not mention Hartnup as a diagnosis one concluded tubular damage, one MSUD and one CPS/NAGS deficiency.
The differential diagnoses of abnormal amino acid excretion patterns due to transport defects is as follows:

Hyperaminoaciduria types	Neutral AA	Pro OHPro	Cys	Lys/Arg/Orn	Gly
Tubular damage*	+	+	+	+	+
Hartnup	+	-	-	-	-/+
Cystinuria	-	-	+	+	-
LPI	-	-	-/+	+	-
Iminoglycinuria	-	+	-	-	+

Suggestions for further investigations included:

- Repeat urine amino acid analysis (provide fresh/clean urine sample)
- Plasma amino acid determination (some participants mentioned tryptophan in particular)
- SLC6A19 mutation analysis (in case no mutation is found: also the partner protein encoded by TMEM27 should be investigated)
- Test siblings (Hartnup disease may be asymptomatic)

Suggestions for treatment were: limit sun exposure to prevent pellagra/photodermatitis, nicotinamide or niacin (vitamin B3) suppletion and a high protein diet.

Many participants commented on the fact that this sample showed evidence of deterioration/bacterial contamination (high pH, benzoate). This explains elevated glutamic acid due to degradation of glutamine. However, degradation in this sample did not impair the ability to come to the correct diagnosis. Increased excretion of neutral amino acids with normal excretion of proline is indicative of Hartnup disease. This is not a generalised amino aciduria as stated by a few participants.

Overall proficiency (based on points) 93%
Recognition of the pattern of elevated neutral amino acids was essential in this sample.

Sample 2017-E: GM1 gangliosidosis (lysosomal β-galactosidase deficiency; OMIM 230500)

Clinical description: A 5-months old female infant presenting with hypotonia, dysmorphic features and arrest of neurological development.

Diagnosis was confirmed by deficiency of lysosomal β-galactosidase and two mutations in the GLB1 gene.

Analytical.
An abnormal oligosaccharide pattern was noted by 19 participants (Fig. 2). One lab stated that oligosaccharides were normal (analysis by LC-MS/MS) and one lab did not perform oligosaccharide analysis, but recommended to do this.
Many participants noted elevations of various organic acids, mostly related to TCA cycle including: fumaric (n=13), adipic (n=9), succinic (n=8), 2-ketoglutaric (n=7).

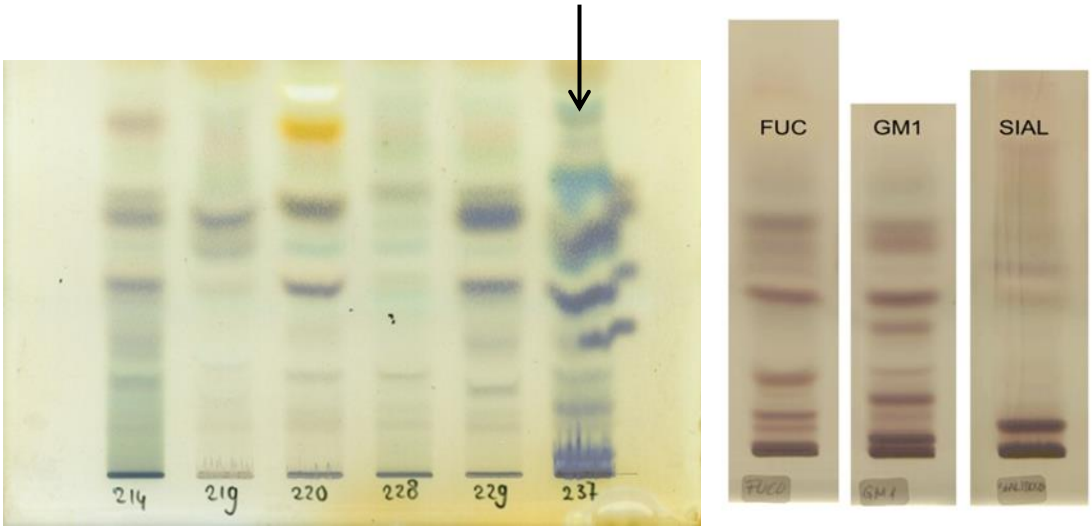

Fig. 2. Oligosaccharide analysis by TLC (left panel). The arrow indicates sample 2017-E. For comparison, a fucosidosis sample, another GM1 sample and a sialidosis sample are also depicted (right panel).

Interpretation.
Fifteen participants interpreted the oligosaccharide pattern as characteristic for GM1. Interpretation of the oligosaccharide pattern was apparently easier compared to the fucosidosis sample included in the 2016 survey (7 correct diagnoses). The following diagnoses were reported:

Diagnosis	most likely	other possible
GM1 gangliosidosis	15	1
Sialidosis/galactosialidosis	2	3
Fucosidosis	1	1
Fumarase def/mitochondrial disease	2	1
No diagnosis	1	-
MPS IV B	-	1

Recommendations focused at measuring β-galactosidase activity (n=13; in leukocytes, fibroblasts or dried blood spot) or other lysosomal enzyme activities consistent with the conclusions reported. Mutation analysis of the GLB1 gene was also recommended by many labs (n=14).
If interpretation of the oligosaccharide pattern is uncertain, the urine should be analyzed next to samples with established oligosaccharidoses (Fig. 2). Please note that an Oligosaccharide kit containing positive urine samples is available at SKML (MCA laboratory, Winterswijk, The Netherlands).

A few reports have appeared in the literature describing oligosaccharide analysis by MALDI-TOF. Recently, Piraud et al have described an LC-MS/MS assay to diagnose oligosaccharidoses (Rapid Commun Mass Spectrom 2017, 31(11):951-963. doi: 10.1002/rcm.7860.

Overall proficiency (based on points) 88%
Most participants noted abnormal oligosaccharides and interpretation of the pattern was well performed.

Sample 2017-F: Lysinuric Protein Intolerance (SLC7A7 deficiency; OMIM 222700)

Clinical description: This boy was referred at the age of 4 y because of refusal to eat, vomiting, short stature and multiple bone fractures.
The urine sample circulated in this survey was the first urine sample obtained from this patient and was sampled before start of any therapy. Plasma amino acid analysis a few days earlier gave values very suspect of LPI: Lys 21 µmol/L, Arg 7, Orn 8, Gln 1730 and Ala 943. Blood NH3 at that time was 53 µmol/L. Diagnosis was confirmed by two different nonsense mutations in the SLC7A7 gene.

Analytical.
Elevated lysine was reported by 17/21 participants, whereas 4 labs considered lysine normal. Raised arginine and ornithine were noted by 12 and 5 labs respectively. High glutamine was reported by 7 labs. 15/21 participants stated elevated orotic acid, but 2 labs listed normal orotic acid. The following excretions were reported (all mmol/mol):

	median	range	n
Lysine	384	314-2472	17
Arginine	29	20-175	10
Ornithine	7	6-41	9
Glutamine	247	34-344	7
Orotic acid	10	(PuPy assay)	6
	22	(Organic acids)	2
	8	(other)	10

Interpretation.
The following diagnoses were reported:

Diagnosis	Most likely	other possible
LPI	15	1
OTC/other UCD	1	3
HFI/fructosemia	1	2
Hypophosphatasia	1	3
No diagnosis	2	-
Osteogenesis imperf	1	-

Most labs concluded LPI. One participant suggested OTC as a diagnosis, but did not recommend to perform plasma amino acid analysis to investigate this diagnosis. Fructose intolerance was reported as the most likely diagnosis by one participant and as a possible other diagnosis by another 2. This relates to the elevated excretion of fructose reported by 2 labs. The meaning of this observation is unclear. The patient did not suffer from hypoglycemia or hepatomegaly. Hypophosphatasia was also stated as a possible diagnosis. The excretion of phosphoethanolamine was normal though.
Suggestions made for further investigations included determination of plasma amino acids (n=14), blood ammonia (n=10) and SLC7A7 mutation analysis (n=13)

Overall proficiency (based on points) 80%
In this urine sample, LPI was not very easy to diagnose; 5 out of 21 participants missed the diagnosis. In 2013 another LPI sample was circulated (2013-F). Overall proficiency was 89% then. Higher proficiency in 2013 may relate to a higher orotic acid excretion, which was 55 mmol/mol (median value) in sample 2013-F against 9 mmol/mol in sample 2017-F. The lysine excretion was comparable in the 2 samples: 358 mmol/mol in 2013-F and 384 mmol/mol in 2017-F. This shows that abnormalities in LPI urine samples may vary widely and that plasma amino acid analysis is needed.

8. Preview of the 2018 scheme and changes in scheme organisation

Samples may be classed as 'educational' in exceptional cases, e.g. when the metabolite pattern in a sample is particularly challenging and diagnosis is hard to reach or when non-standard methods are required. The Scientific Advisory Board decides whether a sample is educational. When a sample, that has been classed educational in an earlier survey, is circulated again it will be scored routinely and cannot be educational again.

The format and logistics of the DPT-NL scheme in 2018 will be identical to 2017.

Tentative planning:

Shipment of samples by CSCQ (all six samples will be dispatched in one box):	February 5, 2018
Analysis start survey 1:	February 26, 2018
Deadline for reporting results of survey 1:	March 19, 2018
Interim report survey 1 available:	April 2018
Analysis start survey 2:	May 28, 2018
Deadline for reporting results of survey 2:	June 18, 2018
Interim report survey 2 available:	July 2018
Discussion of results (ERNDIM workshop Athens t.b.a.)	September 4, 2018
Annual report 2018	December 2018

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