ERNDIM
Diagnostic Proficiency Testing
DPT Centre: Netherlands

ANNUAL REPORT 2014

Scheme Organiser	Scientific Advisor	Deputy Scientific Advisor
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1. Introduction

In 2014 DPT-NL scheme organisation was performed for the first time by CSCQ, the Swiss EQA organisation. The minimal required test panel for participation in any DPT scheme includes dip sticks, amino acids, organic acids and quantitative GAG. DPT-NL additionally requires the analysis of oligosaccharides and purines-pyrimidines. It is strongly recommended to have the following tests available for DPT-NL: qualitative GAG analysis (electrophoresis/TLC), sialic acid, creatine-guanidinoacetate and polyols-sugars. Please note that in DPT schemes it is allowed to obtain results from neighbouring laboratories if one does not offer a certain test, while such test is deemed necessary for a sample. It is required to indicate in the report that results were obtained from a cluster lab.

2. Participants

The 2014 scheme had 20 participating laboratories with the following allocations: Table 1. For both surveys 19 participants submitted results.

Table 1. Participants in DPT-NL 2014

Country	Number of participants
Australia	1
Belgium	5
France	1
Germany	2
The Netherlands	9
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 provides the sources of the samples. Pre-treatment (thiomerosal addition and 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; 2014-1 (samples A, B, C) starting March 17, and 2014-2 (samples D, E, F) starting June 2. Before dispatch to participants one set of samples was sent to the Scientific Advisor and checked. In all six samples the typical metabolic profiles were preserved. Sample dispatch was done March 3, 2014 by DHL. All participants had received the samples by March 10.
Reports of the samples were submitted electronically on 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.
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Table 2. Source of the samples

Sample	Diagnosis	Provider
A	Propionic acidemia	Dutch patient organisation, VKS
B	No IEM	Erasmus Medical Centre, Rotterdam, NL
C	Hypophosphatasia	Dutch patient organisation, VKS
D	MPS III B	Maastricht University Medical Centre, NL
E	OTC female	Maastricht University Medical Centre, NL
F	HHH syndrome	Prof Fowler, Zurich, CH (this was the common sample used in all DPT schemes)

4. Scoring of results

General scoring criteria are depicted in Table 3. Scoring of the 2014 samples was performed according to the criteria summarised in Table 4. In order to achieve harmonised scoring throughout the five European DPT schemes, the ERNDIM Board has instituted a second scoring officer belonging to one of the partner DPT schemes as of 2011. The external scores will be discussed with the scheme's own scientific advisor(s). For the DPT-NL scheme, additional scores were made by the scientific advisor of the DPT UK scheme in 2014.

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 2014 samples.

Sample	Analytical	points	Interpretation	points
A	Abnormal organic acids with typical PA metabolites identified	2	Propionic acidemia	2
B	Normal test results	2	No IEM	2
C	Elevated phosphoethanolamine	2	Hypophosphatasia	2
D	Elevated total GAG Elevated heparansulfate	1 1	MPS III	2
E	Elevated orotic acid	2	OTC UCD other or not specified	2 1
F	Elevated homocitrulline Elevated orotic acid	1 1	HHH syndrome UCD other or not specified	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' will be 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 at the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on March 19, 2015. The following possible critical errors were identified in the 2014 scheme (Table 5).

Table 5. Possible critical errors in the 2014 scheme.

Sample	Critical error	No. of occurences
A	not reporting PA	0
B	none	-
C	none	-
D	none	-
E	not reporting orotic acid	0
F	not reporting orotic acid or any UCD	1

5. Communication of results

This year we were able to use the evaluation programme to generate individual lab reports and these were distributed on May 14th and July 25th. These individual participant reports included the scores obtained.
Discussion of the results took place in Innsbruck during the ERNDIM workshop held at the SSIEM conference on September 2, 2014 (for the minutes of the meeting: see item 9, below). The meeting, as usual open to participants only, was attended by representatives from 11 of the participating institutes. George Ruijter, Erasmus Medical Centre Rotterdam, chaired the meeting and made a presentation of the analytical/diagnostic points of interest. This presentation has been sent to all DPT-NL participants. In addition, analysis of the results submitted and items discussed during the DPT meeting are part of the Annual Report
Finally this annual report summarises scheme organisation and results.

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

One Performance Support letter will be send for the 2014 surveys. Two were sent in 2013.

6. Proficiency of the 2014 surveys

Proficiencies (% of maximal achievable points for all labs) of the 2014 samples are summarized in Table 6. Distribution of scores is given in Table 7.
Maximal.scores (24 points) were obtained by 4 out of the 19 participating labs. Samples A, B and C were straightforward, while sample D, E and F were more challenging. Overall performance for all six samples was 85%, considerably better than previous years.

Table 6. Performance on the DPT 2014 samples.

Sample	Diagnosis	No. of reports	Proficiency (%)		
			analytical	interpretation	TOTAL
A	Propionic acidemia	19	100	100	100
B	No IEM	19	95	84	89
C	Hypophosphatasia	19	89	89	89
D	MPS III B	19	68	66	67
E	OTC female	19	100	71	86
F	HHH syndrome	19	79	82	80

Table 7. Distribution of final scores; for each sample the number of participants with score 0/1/2/3/4 points is given.

Sample	0 points	1	2	3	4
A	0	0	0	0	19
B	1	0	2	0	16
C	2	0	0	0	17
D	4	3	0	0	12
E	0	0	5	1	13
F	0	1	4	4	10

7. Results of individual samples and evaluation of reporting

Sample 2014-1A: Propionic academia (OMIM 606054).

Clinical description: This girl was referred to the hospital 3 days after birth with kussmaul breathing, weight loss and low temperature. The sample was collected at age 8 years.

Sample A was a straightforward sample that was correctly identified by all participants. The following characteristic metabolites were reported most frequently:

	n(quan)	n(qual)	median mmol/mol	min - max mmol/mol
Propionylglycine	11	18	220	1 - 901
Tiglylglycine	10	16	142	63 - 598
3-Hydroxypropionic acid	13	17	1175	73 - 4231
Methylcitric acid	10	17	889	194 - 1555
3-Hydroxyisovaleric acid	4	8	54	45 - 183
3-OH-butyric acid	6	7	41	16 - 89

Of the 7 labs that reported 3-OH-butyric acid, 4 interpreted the level as elevated, while 3 reported 'normal'.
Other organic acids reported were: fumaric, malic, succinic, methylmalonic, adipic, lactic, glutaric, propionic, acetoacetic. 4-methylpimelic, 2-methyl-3-OH-butyric, 2-methyl-3-keto-butyric, 3-OH-n-valeric and 3-methylglutaconic acid. 4-Methylpimelic acid is produced by fatty acid biosynthesis using propionyl-CoA as a substrate instead of AcCoA (Jacobs et al, 1984, Pediatr Res 18, 1185). The 3-methylglutaconic acid reported is probably miss-identified 2-methylglutaconic acid (Duran et al, 1982,Biomed Mass Spectrom 1,1-5).
Multiple carboxylase deficiency (MCD) was considered possible by 2 labs, while 5 participants reported this to be unlikely. According to reports in the literature (e.g. Suormala et al, 1997, Pediatr Res 41, 666-673) the MCC metabolites 3-OH-isovaleric acid and 3-methylcrotonylglycine are the most abundant compounds in (biotin-responsive) MCD. This is not the case in sample A and MCD is therefore unlikely.
As expected elevated glycine was reported by most participants (n=17, Median 2709 mmol/mol, min – max 759 – 4345 mmol/mol).

Further investigations reported:

plasma amino acids	8
plasma/serum acylcarnitines	11
PCC activity in leu/fib	15
PCCA, PCCB mutations	16

It was suggested by dr Sass to proceed immediately with mutatioanalysis and to skip determination of enzyme activity.

Sample 2014-1B: No Inborn error of metabolism.

Clinical description: A 12 year-old male with psychomotor retardation.

Most participants reported normal results for this sample (16). Two participants did report a diagnosis. One concluded sulphite oxidase deficiency based on the presence of S-sulfo-cysteine. Another lab reported a possible creatin transporter deficiency, presumably based on the clinical description since creatin was reported normal by this participant. One lab did not enter any results under interpretation.

Results were reported for the following tests:

DPT minimal panel	n	Other	n
Creatinine	19	Purines-pyimidines	16
Dipstick	16	Oligosacharides	17
Aminoacids	19	Gua/Cre	15
Organic acids	19	Sialic acid	11
GAG quant	19	GAG electrophoresis	5
		Acylcarnitines	5
		Sulphite	5
		Pipecolic acid	4
		Reducing substances	3
		Polyols	3
		Bile acids/alcohols	2
		Pterins	1
		Phenolic acids	1

The requirement for recommendations in a non-IEM sample was discussed during the meeting. The general consensus was that recommendation are not required. The ERNDIM Scientific Advisory board has also discussed this and came to the same conclusion.

Sample 2014-1C: Hypophosphatasia (OMIM 241510).

Clinical description: A girl, 4 years of age, with premature loss of primary teeth and waddling gait. The urine sample was collected at age 18 years.

Seventeen labs (89%) correctly identified elevated phosphoethanolamine (PEA) and concluded hypophosphatasia. Two participants did not report elevated PEA. One lab reported MPS IV as a possible diagnosis based on elevated total GAG and abnormal electrophoresis results. The second lab that missed elevated PEA did not report a diagnosis.
Although this was a relatively straightforward diagnosis, the Scientific Advisory board decided that this sample was not eligible as a critical error, since PEA determination is not the best method to establish hypophosphatasia.
PEA (n=17) values reported were: Median 51 mmol/mol, Mean 48 mmol/mol, SD 14, Min – max 19 – 79. See Fig. 1 for an example amino acid chromatogram of sample 2014-1C.

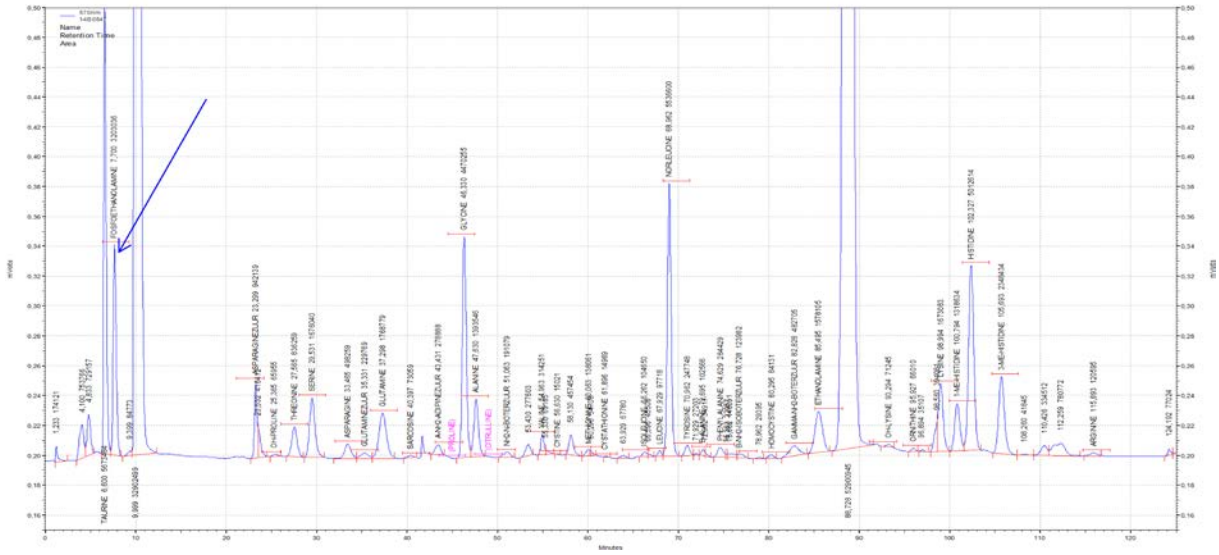


Fig. 1. Amino acid profile of sample 2014-1C using Biochrom 30. The arrow indicates PEA, which elutes directly after taurine.

Various phenotypes have been reported for hypophosphatasia: perinatal (lethal), infantile, childhood and an adult form, which might include heterozygote individuals. Another description of the milder phenotype is odontohypophosphatasia. Currently treatment by ERT is explored.

The following further investigations were reported:

Serum/plasma alkaline phosphatase activity	16
ALPL mutation analysis	16
Serum/plasma pyridoxal-P	11
Bone X-ray	4

Also in 2006 a hypophosphatasia sample was circulated (DPT-NL 2006-F). Proficiency in 2006 was 72% with 13 out of 18 correct diagnoses. Proficiency in the current sample is higher, but it must be noted that this was a different sample.

Patient 2014-2D: Mucopolysaccharidosis type III B (OMIM 252920).

Clinical description: An adult, retarded, woman with psychiatric problems, retinitis pigmentosa and brain atrophy. No dysmorphic features were noticed.

This sample was challenging for two reasons. First, the clinical description was not typical for a mucopolysaccharidosis patient and secondly, the total GAG excretion observed with this mild adult patient was apparently not clearly elevated. Proficiency was 63%. This sample was also circulated in 2008 (DPT NL 2008-1A). In 2008, 6 out of 20 labs came to the correct diagnosis (30%), while two labs reported ´MPS unspecified´. Proficiency has clearly increased. It is tempting to speculate that this positive change might be attributable to participation in proficiency testing schemes.

Quantitative GAG results were reported by 18 labs. Total GAG was reported elevated by 13 labs (all using DMB assay), whereas five labs reported a normal value for total GAG (4 using DMB, one using Harmine). A large range of GAG values was reported: 4-12 mg/mmol. The distribution of values reported is depicted in Fig. 2 and clearly shows that ´normal´ test results (false-negative, shown in red) are all relatively low values. However, amongst the lower values about an equal number of labs interpret the GAG test result as elevated. This shows that definition of reference values is critical for the quantitative GAG test.
Some data on the GAG type used by participants as a calibrator in the quantitative GAG test were available (n=8). Four labs used CS as a standard: 2 found GAG elevated in this sample and 2 normal. The 4 labs known to use HS as a standard all interpreted the GAG level in sample 2014-2D as elevated. This might suggest that HS is a superior calibrator to identify mild MPS III patients in the DMB test. The number is small, however, and a larger sample size is required to confirm this conclusion.
Fifteen labs performed GAG subtype analysis (e.g. by electrophoresis), 12 found elevated HS.

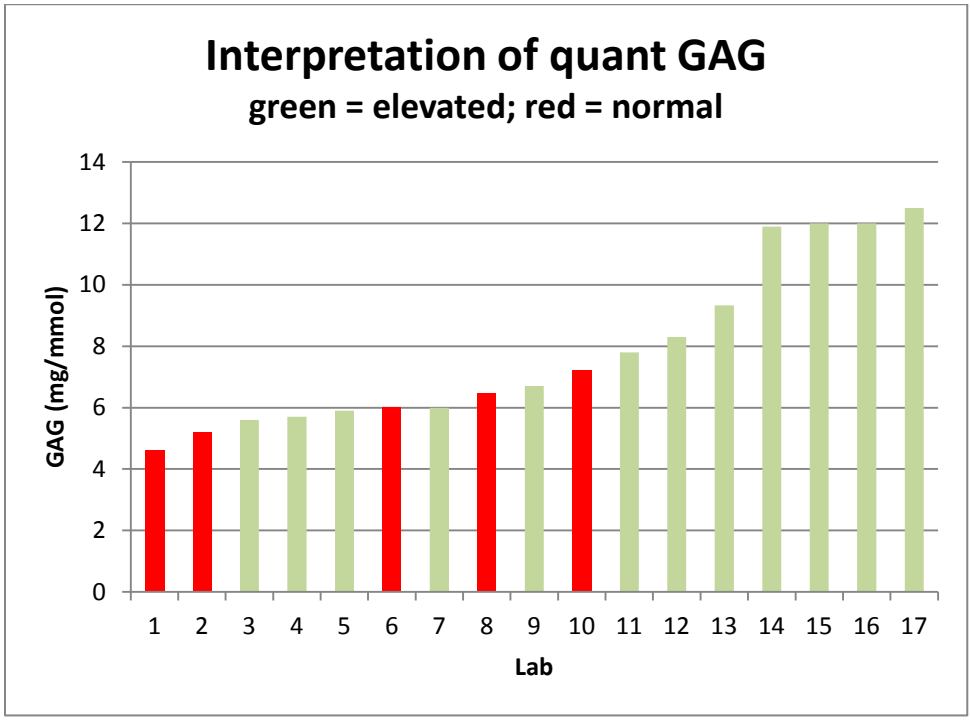


Fig. 2. Distribution of quantitative total GAG test results reported by participants and interpretation of results according to (local) reference values. Note: one value reported was 85 mmol/mol and not included in the figure.

Sample 2014-2E: Ornithine transcarbamylase (OTC) deficiency (OMIM 311250); female with 15% residual acitivity

Clinical description: A girl aged 1.5 years with skin rashes, total malaise, increased liver enzymes and disturbed coagulation parameters

Twelve participants reported OTC deficiency as the most likely diagnosis; one concluded HHH syndrome. The strongly elevated concentration of orotic acid was detected by all participants and led 6 labs to conclude UMPS deficiency. One of these mentioned that a urea cycle defect also was a possibility. The clinical symptoms were clearly not characteristic for UMPS deficiency. In addition, treatment was not mentioned in the clinical description. For DPT schemes, specific treatment that might influence test results and accordingly conclusions, will be mentioned in the clinical description. Clues to the diagnosis OTC deficiency were elevated glutamine, uridine and uracil. In untreated UMPS deficiency uridine and uracil are not expected to be present. The accumulation of pyrimidine metabolites in an OTC patient has been described by Van Kuilenburg et al, 2006, Nucleosides Nucleotides and Nucleic Acids 25, 1251-1255 (Fig. 3).

Orotic acid concentrations reported:

Method	OA	PuPy	Special
n(quan)	7	9	10
n(qual)	18 (all ↑)	17 (all ↑)	11 (all ↑)
Median	3834 mmol/mol	2693	2242
Mean	4977	3889	2125

Other pyrimidines

	Uracil	Uridine
n(quan)	12	9
n(qual)	17 (all ↑)	10 (all ↑)
Median	880 mmol/mol	361 mmol/mol
Mean	817	351

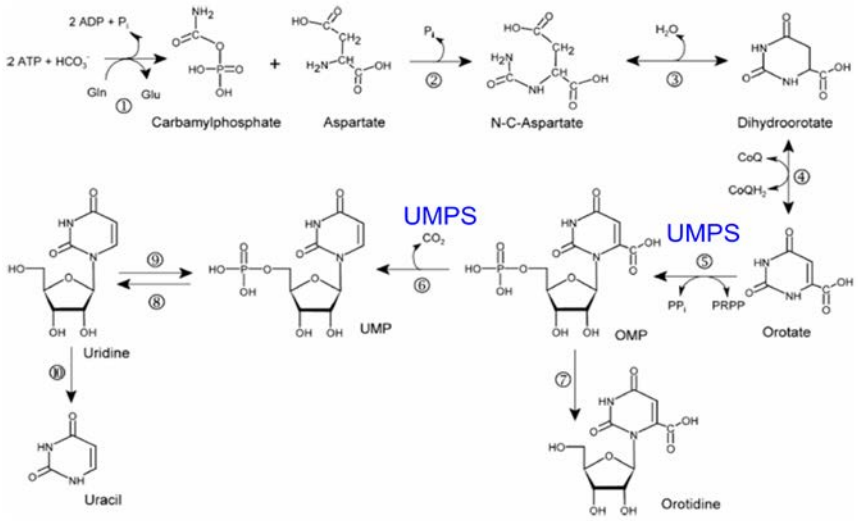


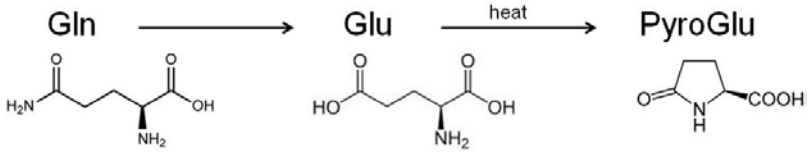
FIGURE 1 Pyrimidine de novo pathway. ①, carbamylphosphate synthetase; ②, aspartate transcarbamylase; ③, dihydroorotase; ① + ② + ③, CAD; ④, dihydroorotate dehydrogenase; ⑤, orotate phosphoribosyltransferase; ⑥, orotidine 5'-monophosphate decarboxylase; ⑤ + ⑥, UMP synthase; ⑦, orotidine 5'-monophosphate phosphohydrolase; ⑧, pyrimidine 5' nucleotidase; ⑨, uridine kinase; ⑩, uridine phosphorylase.

Fig. 3 (Fig.1 from Van Kuilenburg et al, 2006, Nucleosides Nucleotides and Nucleic Acids 25, 1251-1255)

Data of relevant amino acids were as follows:

	Glutamine	Homocitrulline
n(quan)	11	5
n(qual)	15 (11 ↑; 4 normal)	6 (all ↑)
Median	297 mmol/mol	43 mmol/mol
Mean	290	45
SD	32	4
min – max	238 – 327	40 – 51

Glutamine in urine was not clearly elevated; 4 out of 15 labs reported normal glutamine. Glutamine can be converted to pyroglutamate upon heating and interestingly 2 labs reported elevated pyroglutamate (mean value 231 mmol/mol).



Six labs found elevated homocitrulline in sample 2014-2E and this may have led to some confusion as another sample in survey 2014-2: sample F turned out to be HHH syndrome (this was the common sample). Homocitrulline can be the product of the OTC reaction using lysine as a substrate instead of ornithine (Fig. 4). This has been reported for patients with HHH syndrome or hyprlysinemia. With OTC deficiency, this is not expected to occur in patient 2014-2E. Another source of homocitrulline that has been suggested is heat-treated milk. This may very wel be the case in the 18 month-old patient E. Since HHH syndrome cannot be distinguished from OTC in this sample, the diagnosis HHH syndrome has also been scored with full points.
Analysis of homoarginine may help to distuinguish exogenous homocittruline from OTC-derived homocitrulline. In HHH syndrome as well as hypelysinemia, homoarginine is present, whereas it is not in OTC deficiency (Fig. 4 and 5).
Relevant recommendations for further research reported were: plasma amino acid analysis (n=8) and OTC mutations (n=10).

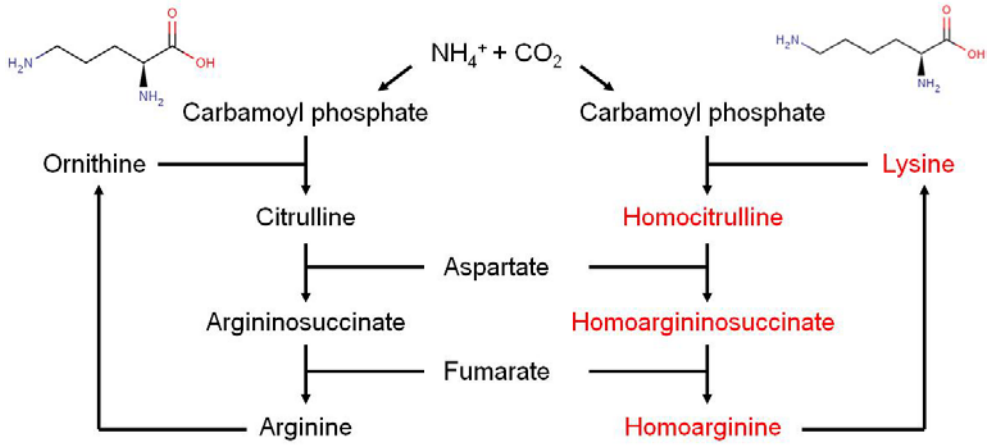


Fig.4. Production of homocitrulline in hyperlysinemia and HHH syndrome.

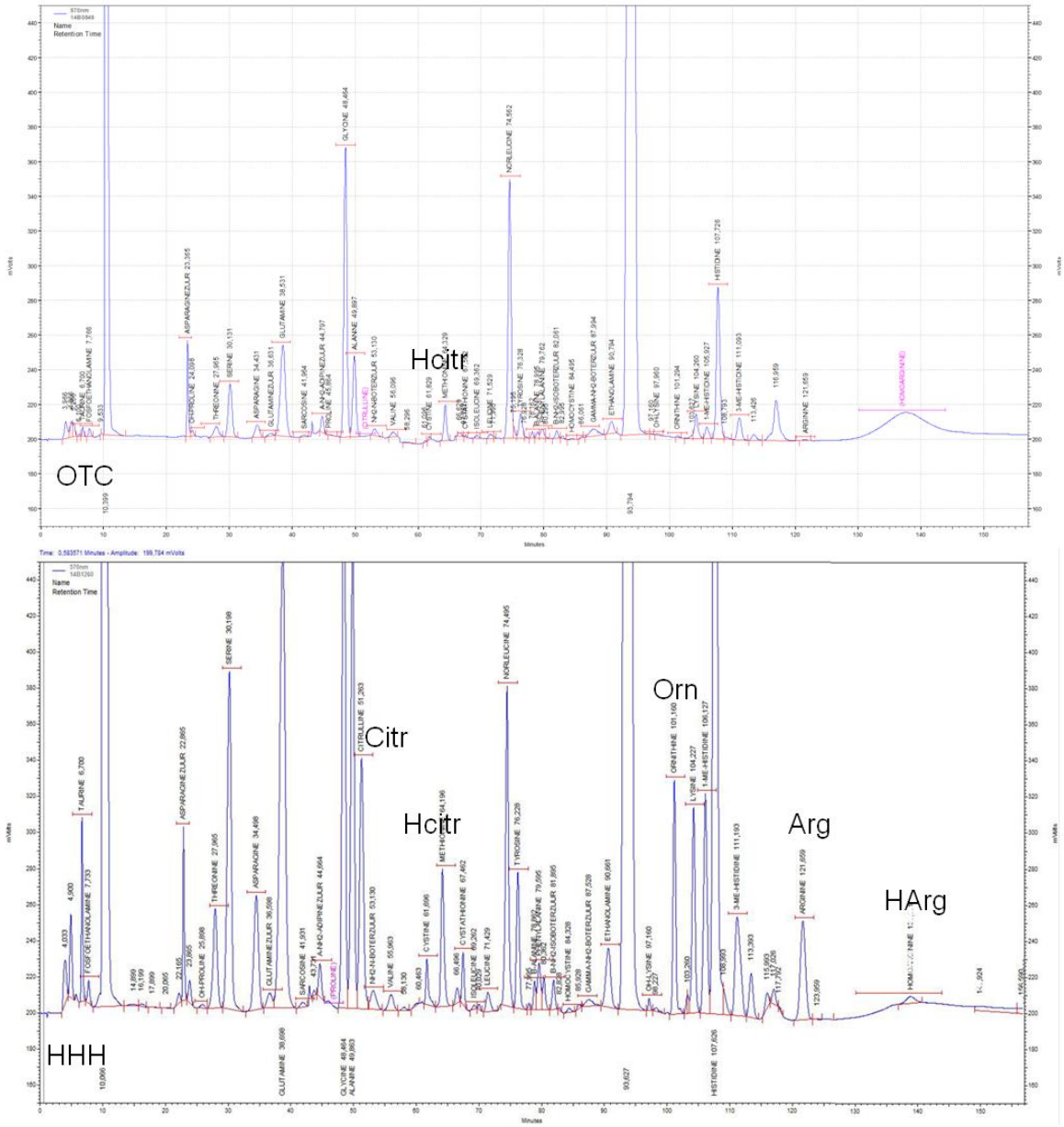


Fig.5. Amino acid chromatograms of samples 2014-2E (top) and 2014-2F (bottom).

Sample 2014-2F: HHH syndrome (OMIM 238970)

Clinical description: Following uneventful pregnancy and birth this male child showed mild hypotonia at 6 months of age. A few months later, developmental delay and failure to thrive with elevated transaminases was observed. The urine was collected at the age of 8.75 years whilst receiving specific treatment.

Sample F was the common sample and was provided by DPT Switzerland. Results were discussed by Prof Brian Fowler during the ERNDIM Workshop in Innsbruck on September 2, 2014. The presentation is available at the ERNDIM.ORG website.
The common sample was from a patient suffering from HHH syndrome. In DPT-NL, all but 1 lab found elevated orotic acid, while 12 labs reported elevated homocitrulline. Twelve laboratories mentioned HHH syndrome as a possibility, while 7 concluded other UCDs (citrulinemia type I, type II or OTC). Proficiency in DPT-NL was 80%, while overall proficiency for all DPT schemes was 69%

8. Preview of the 2015 scheme

The format and logistics of the DPT-NL scheme in 2015 will be identical to 2014.

Tentative planning:

Shipment of samples by CSCQ (all six samples will be dispatched in one box):	March 30, 2015
Analysis start survey 1:	April 7, 2015
Deadline for reporting results of survey 1:	April 28, 2015
Interim report survey 1 available:	May 28, 2015
Analysis start survey 2:	June 1, 2015
Deadline for reporting results of survey 2:	June 22, 2015
Interim report survey 2 available:	July 31, 2015
Discussion of results (ERNDIM workshop at SSIEM symposium, Lyon):	September 1, 2015
Annual report 2015	April 2016

9. Minutes of the ERNDIM DPT NL 2014 discussion

Date & time: September 2, 2014, 9.00 – 10.30
Location: Innsbruck Congress Center, room Freiburg Nord (3rd floor)

Attendants: G Salomons (Amsterdam), L Kluijtmans (Nijmegen), D Habets (Maastricht), J Jans (Utrecht), B Prinsen (Utrecht), F Eyskens (Antwerp), P Burda (Zurich), J Sass (Zurich) R Heiner (Groningen), W Onkenhout (Leiden), G Martens (Brussels), S Marie (Brussels), N Abeling (Amsterdam), G Ruijter (Rotterdam, chair)

Absent with notification: L Greed (Perth), M Dercksen (Potchefstroom), C Saban (Lyon), C Aquaviva (Lyon), M Wamelink (Amsterdam), MF Vincent (Brussels)

1. Welcome.
2. The agenda was not modified.
3. The minutes of the meeting in Barcelona on September 3, 2013 (embedded in the 2013 DPT Amsterdam annual report; http://cms.erndimqa.nl/) were approved.
4. News from ERNDIM is provided by the chair of ERNDIM (dr Mick Henderson during the general part of the ERNDIM workshop.
5. Logistics:
   - The tests panel required for participation in the DPT schemes was reiterated (see also item 1 of the 2014 annual report).
   - Shipment of samples was performed for the first time by CSCQ in 2014. No letter was accompanying the samples apart from a delivery note. Some labs were confused by the delivery note stating that samples should be stored at 4 deg C. In 2015 a separate information letter will be included in the package. As a general note, labs are responsible for sample storage after receipt; samples should be treated according to local procedures just as any other sample received for diagnostic purposes. In response to a request made by several participants, age and gender of the 'patients' will be made available at the time of sample dispatch in order to facilitate registration of the sample in a LIMS system upon reveipt (i.e. before the analysis start date).
   - Participants are requested to provide urine samples, minimum 300 mL. This will give you a 20% discount for the DPT scheme in the year following utilization of the sample in the scheme. For the common sample, 1.5 L is required. Please contact the scientific Advisor when you have a sample available.

- Website reporting worked well in 2014. One minor point is the item ‘Date of reporting results’ in the proof reading document. This is actually the date of downloading the proof reading doc. This bug will be communicated to CSCQ.
- Interim reports are appreciated very much.

6. Planning and organisation of DPT-NL 2015: see also item 8 of the 2014 annual report
7. Any other business: none
8. Discussion of the 2014 samples A-B-C-D-E (F was the common sample). Details are provided in item 7 of the 2014 annual report.
9. Date and time of the next meeting: September 1, 2015 in Lyon.

Rotterdam, April 4, 2015

Dr George Ruijter
Scientific Advisor

Note: This annual report is intended for participants of the ERNDIM DPT-NL scheme. The contents should not be used for any publication without permission of the scheme advisor