ERNDIM
Diagnostic Proficiency Testing
DPT Centre: Netherlands

ANNUAL REPORT 2013

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1 Introduction

Diagnostic Proficiency Test (DPT) schemes for inborn errors of metabolism organised by ERNDIM continue to be the ultimate challenge for biochemical genetics labs. Since 20 years this scheme has been run in conjunction with SKML, the Dutch QA-organization for medical laboratories with Dr.Cas Weykamp acting as scheme organiser. As of 2014 scheme organisation will be performed by CSCQ, the Swiss EQA organisation. Cas Weykamp and his staff, in particular Irene de Graaf, are sincerely acknowledged for their active contributions to DPT-NL.

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 2013 scheme had 19 participating laboratories with the following allocations

Country	Number of participants
Australia	1
Belgium	5
Germany	1
The Netherlands	10
Oman	1
South-Africa	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 1 provides the sources of the samples. Pre-treatment (thiomerosal addition and heat-treatment) and dispatch of the samples was done by the Scheme organiser. Two surveys were performed; 2013-1 (samples A, B, C) in February and 2013-2 (samples D, E, G) in June/July. Before dispatch samples are checked by the Scientific Advisor. Sample dispatch was done by regular mail. As discussed before, this may cause some delay for laboratories outside Europe. This potential drawback has been overcome by sending the samples to the Australian, Omani and South African participants two weeks prior to the regular shipment.
Reports of the samples were submitted electronically on the website of the Swiss organisation for quality control (CSCQ) (https://baal.hcuge.ch/cscq/ERNDIM/Initial/Initial.php). In line with previous discussions the time allotted for submitting reports was 4 weeks with the date of shipping the samples as starting point.
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 Lyon scheme in 2013.
Discussion of the results took place in Barcelona during the ERNDIM workshop held at the ICIEM conference on September 3, 2013 (for the minutes of the meeting: see item 7, below). The meeting, as usual open to participants only, was attended by representatives of most 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.

Table 1. Source of the samples

Sample	Diagnosis	Provider
A	Methylmalonic acidemia	Dutch patient organisation, VKS
B	MNGIE	Erasmus Medical Centre, Rotterdam, NL
C	Cerebrotendinous Xanthomatosis	AMC, Amsterdam, NL
D	OAT deficiency (Gyrate atrophy)	SKML DPT sample collection
E	Aspartylglucosaminuria	Maastricht University Medical Centre. NL
G	Lysinuric Protein Intolerance	Dr Kozich, Prague, CZ (this was the common sample used in all DPT schemes)

4 Scoring of results

Table 2. 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 3. Specific criteria for scoring results of the 2013 samples.

Sample	Analytical	points	Interpretation	points
A	Elevated mma	2	MMA	2
B	Elevated thymidine and/or deoxyuridine (and thymine, uracil)	2	MNGIE	2
	Elevated thymine and/or uracil	1	Mitochondriopathy	1
D	Elevated ornithine	2	Gyrate atrophy/OAT deficiency	2
E	Elevated aspartylglucosamine and/or AGU oligosaccharide pattern	2	Aspartylglucosaminuria	2
	Abnormal oligosaccharide pattern	1		
G	Elevated lys, arg,orn	1	LPI	2
	Elevated orotic acid	1		

From 2013 onwards the scoring principle has changed. In the past, one extra point could be obtained by identifying the correct additional lab test to confirm the diagnosis e.g. enzyme testing or molecular analysis. This advice is now incorporated in the interpretation part of the report.
The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. Patient C was affected with cerebrotendinous xanthomatosis (CTX), a treatable inherited condition resulting from a defect in bile acid synthesis. Its diagnosis in urine requires the analysis of bile alcohols by (tandem) mass spectrometry. Although many of the participating labs have access to this analytical technique, only a disappointingly small number of labs made the correct diagnosis. It was therefore decided by the SAB to remove this sample from the scoring table. Accordingly, the maximum score for 2013 was set at 20 points. In accordance with a previous decision by the board, participants who failed to achieve satisfactory performance were those who scored less than 12 points out of the maximum of 20 in this year.

Scores have been sent to individual participants by email.
ERNDIM provides a single certificate for all its schemes with details of participation and performance.
Two Performance Support letters will be send for the 2013 surveys. None were sent in 2012.

Starting with the 2014 schemes the concept of 'critical error' will be introduced to the assessment of the DPT schemes (see item 6, Preview of the 2014 scheme).

5 Results of individual samples

Table 4. Performance on the 6 samples.

Sample	Diagnosis	No. of reports	Proficiency (%)		
			analytical	interpretation	TOTAL
A	Methylmalonic acidemia	19	95	97	96
B	MNGIE	19	61	66	63
C	Cerebrotendinous Xanthomatosis	19	32	39	36
D	OAT deficiency (Gyrate atrophy)	19	92	92	92
E	Aspartylglucosaminuria	19	87	79	83
G	Lysinuric Protein Intolerance	18	87	92	89

Table 5. 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	1	1	17
B	5	1	1	3	9
D	1	0	1	0	17
E	1	3	0	0	15
G	1	0	1	2	15

Eight out of the 19 participating labs had the maximal score of 20 points.
The total number of reports was 94 out of the 95 which were expected on the basis of the number of registered participants. For all samples, 73 out of 94 reports (78%) were correct. This result is identical to the overall performance in 2012

Sample 2013-1A: Methylmalonic aciduria.

Clinical description: This male patient was diagnosed at the age of 3 days following referral for feeding difficulties and Kussmaul breathing. The sample was taken at the age of 9 years.

This was a straightforward sample that was correctly identified by 18 of the 19 participants. The median MMA concentration in this sample was 3592 mmol/mol creatinine (n=15; range 630-4800). Methylcitric acid was quantified by 8 labs. The median value was 40 mmol/mol (range 11-241). A commercial methylcitrate standard is available at CDN Isotopes.
Most labs reported normal amino acid concentrations, Glycine was normal in this sample.
Homocyst(e)ine was reported by 5 labs; all found a normal level. Homocyst(e)ine should be measured and specifically reported when mma is elevated.
The following conclusions were reported: 3 x mma, 15 x mma/cbl(AB). The following specifications were reported: 4 x cblCDF possible, 2 x cblCDF unlikely.
Analysis of acylcarnitines (Fig. 1) may provide additional information when moderate elevations of mma are observed, e.g. to distinguish B12 deficiency (mainly C3 increased) from SUCL defects (predominantly C4DC).
Further investigations reported were: plasma homocysteine (13), mutase activity (14), complementation analysis (11) and B12 status (6).

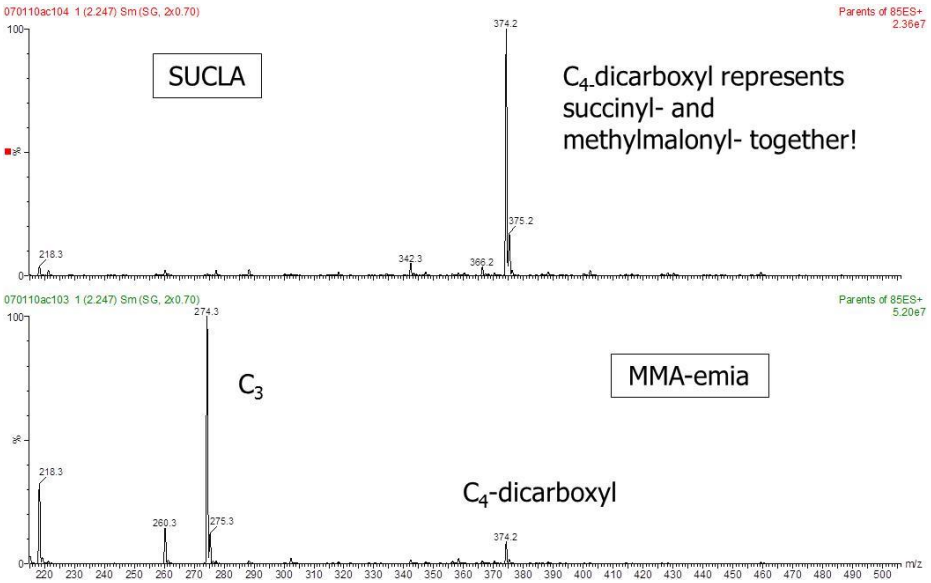


Fig. 1. Acylcarnitine analysis of MMA and SUCLA urine samples

Sample 2013-1B: Mitochondrial NeuroGastroIntestinal Encephalopathy (MNGIE) due to thymidine phosphorylase deficiency.

Clinical description: A 14 year-old female with psychomotor retardation, myopathy and severe cachexia (weight 26 kg). MRI revealed leukodystrophy.

Twelve participants reported MNGIE (63 %; reliability 1-3), 2 mitochondriopathy, 3 labs 3-OH-isobutyric aciduria and 2 'no diagnosis'.
Ten labs reported elevated thymidine and/or deoxyuridine. Median concentrations were 23 mmol/mol (range 21-25) for thymidine and 55 mmol/mol (range 53-60) for deoxyuridine. The ranges of results reported for these two metabolites are very small and dr Jörgen Bierau (Scientific Advisor of the quantitative Purine-Pyrimidine scheme) commented that this consistent with substantially improved performance in the PuPy scheme. Three labs only reported thymine and/or uracil. These latter metabolites are not specific for MNGIE and may indicate defects in pyrimidine degradation, i.e. Dihydropyriminidase or Dihydropyrimidine DH. The source of uracil and thymine, products of the Thymidine Phosphorylase reaction, is not completely clear. Probably Uridine Phosphorylase acts on thymidine/deoxyuridine at high concentrations. Also spontaneous or bacterial degradation of the accumulating metabolites may contribute.
Many labs reported increased levels of various organic acids: in particular lactate, 3OHisobutyric acid and various krebs cycle intermediates (see Table 6). These abnormalities are secondary to mitochondrial failure (see Bennett et al 1993 JIMD 16:560-562).
In this patients various other abnormalities are secondary to TP deficiency: low creatinine due to cachexia and glucosuria, hyperaminoaciduria due to tubulopathy.
The following advices for further investigations were given: thymidine phosphorylase enzyme analysis (10), TYMP mutations (9), lactate (pyruvate) in blood (3), thymidine/ dUridine in plasma (2), muscle biopsy (mtDNA, ETC activity assays) (4) and CSF lactate (1).
The clinical description of this patients was very suggestive for MNGIE syndrome and analysis of pyrimidines should be performed in such a case.

Table 6. Organic acid results reported for sample B (MNGIE).

Centre	lactic	3-OH-isobutyric	3-OH-butyric	malic	Fumaric	succinic	3HIVA	
	773	89	80	67	29			
	↑↑	↑↑	↑				↑	
	462							
	1360							
	↑	↑	↑		↑	↑		thymine
	808		382	67	64			
		↑						Uracil, thymine
	1200	260				62	260	Thymine, 2HIVA
	1606	394	166		44	111	101	Thymine, citric
	↑	↑	↑	↑	↑	↑	↑	MMA, 3OHpropionic
	1700	135	88	34				
	747	155	↑	14	16			Uracil, thymine
	750	↑	120		21			2HIVA, 2OHbutyric
	↑	↑					↑	3OHpropionic
	1061							
	↑	↑		↑	↑		↑	2HIVA, 3OHpropionic, aconitic, ethylhydracrylic
					13.6			
	461	274	↑				↑	3OHpropionic, 2OHbutyric

Sample 2013-1C: Cerebrotendinous Xanthomatosis (CTX)

Clinical description: The 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; they both had a low educational level.

Cataract may result from a number of diffierent IEM. The DD includes RCDP, CTX, Lowe syndrome, defects in galactose metabolism, mevalonic aciduria and mitochondrial defects.
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. 2.

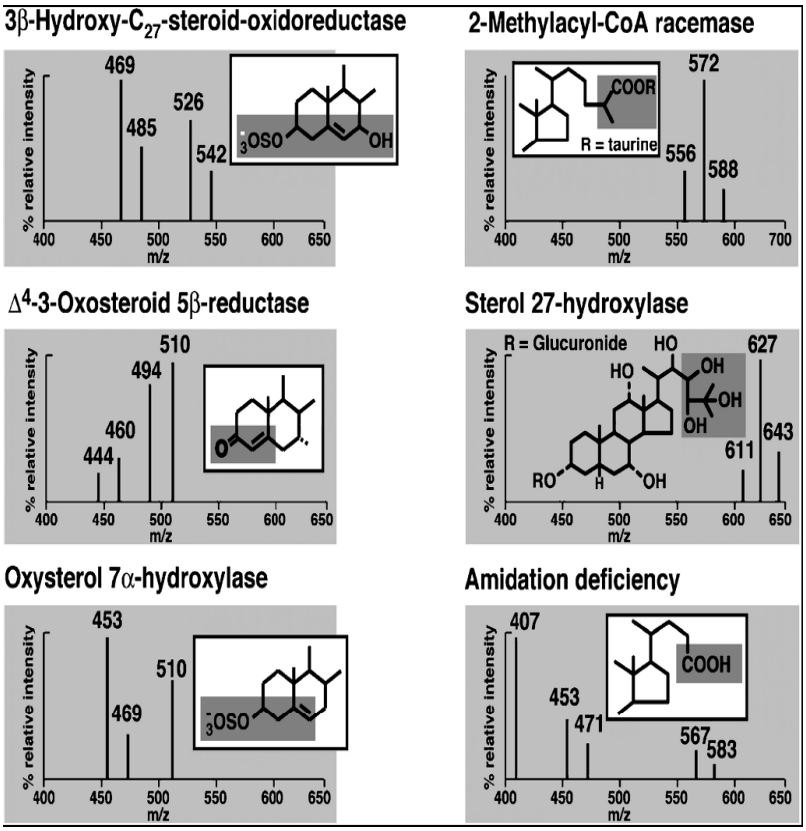


Fig. 2. Bile acid MS profiles of various bile acid biosynthesis defects (from Defects in bile acid biosynthesis--diagnosis and treatment. Setchell KD, Heubi JE.2006 J Pediatr Gastroenterol Nutr. 2006 43 Suppl 1:S17-22).

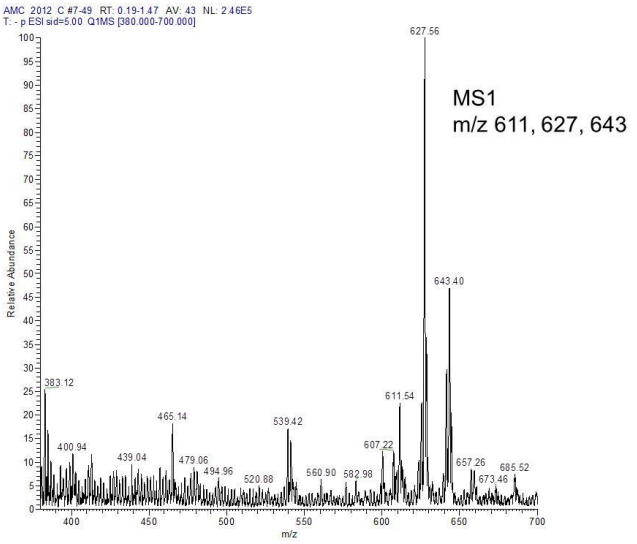


Fig. 3. Typical MS profile of a CTX urine.

Seven labs reported elevated C27 bile alcohols (in particular cholestane pentol glucuronide) and concluded CTX. A typical urine MS profile of a CTX patient is depicted in Fig. 3.
Further investigations reported included determination of cholestanol in plasma and CYP27A1 mutation analysis.
CTX is a treatable disease (suppletion of chenodeoxycholic acid: Chenofalc/Xenbilox) and should be diagnosed by a Biochemical Genetics laboratory..
The proficiency of sample C was low and 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).

Patient 2013-2D: Gyrate atrophy (Ornithine aminotransferase deficiency)

Clinical description: This patient is a 10 year old male suffering from progressive visual loss.

Elevated ornithine was reported by 18 labs and all these participants came to the correct conclusion.

High ornithine in urine may indicate the following IEM:

OAT	aminopiperidon also elevated (all other amino acids normal)
LPI	lys, arg (and sometimes cystine) also elevated
Cystinuria	cystine, lys, arg also elevated
HHH syndrome	homocitrulline also elevated
Hyperlysinemia	lys (and saccharopine) also elevated

Aminopiperidon is a cyclic derivative of ornithine. Detection may depend on the analytical system used. Using Biochrom amino acid analysers it elutes close to 3-methyl-histidine/anserine (Fig 4). The ratio to ornithine is approximately 1/3.
Further investigations reported were: plasma amino acids (9), OAT enzyme activity/mutations(16) and plasma creatine (1).

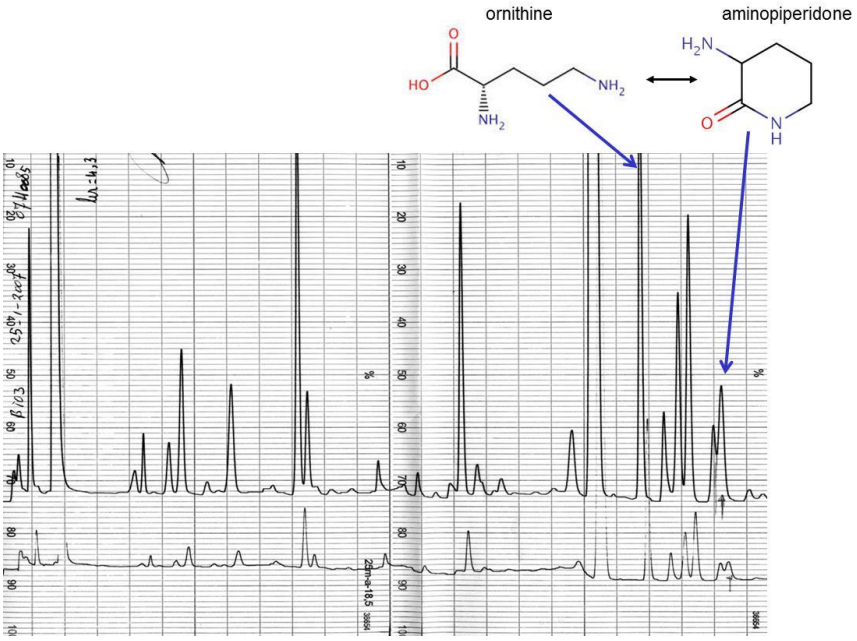


Fig. 4. Amino acid analysis pattern of an OAT urine sample using a Biochrom 30.

Sample 2013-2E: Aspartylglucosaminuria

Clinical description: A slightly dysmorphic 6 year old male with mental retardation, motor retardation and hypotonia.

Twelve labs reported elevated aspartylglucosamine in amino acid analysis. Two participants quantified this metabolite: 257 and 265 mmol/mol. Aspartylglucosamine elutes just before urea in traditional amino acid analysers (Fig. 5). One participant commented that it co-eluted with S-Sulfocysteine in UPLC using C18 chromatography.

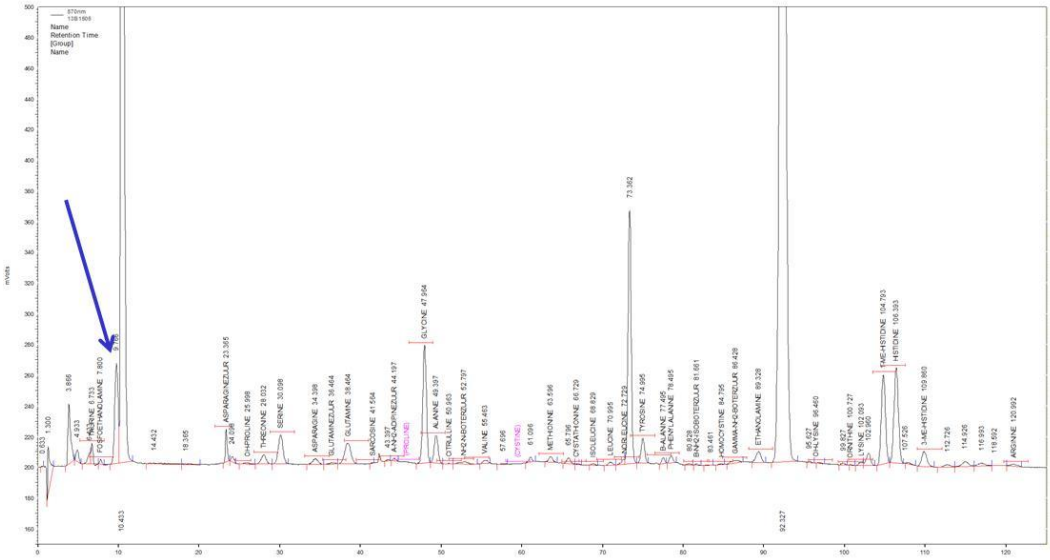


Fig. 5. Biochrom 30 analysis of an Aspartylglucosaminuria urine sample showing the presence of aspartylglucosamine.

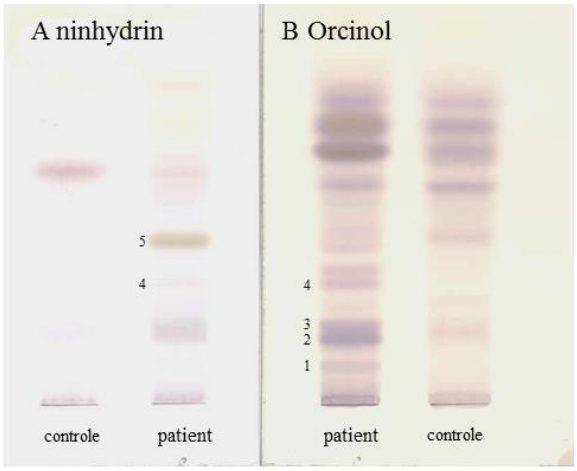
Seventeen labs performed oligosaccharide analysis and all concluded that the profile was abnormal: 15 labs concluded aspartylglucosaminuria, 2 sialidosis/galactosialidosis. Other diagnoses reported were MPS III/sulphite oxidase/MoCo deficiency (1) and Lowe syndrome (1).
An oligosaccharide TLC pattern of this urine is shown in Fig. 5 (kindly provided by dr Kluijtmans and dr Huigen, Radboud University Medical Centre, Nijmegen).

The following advices were given for further investigations: aspartylglucosaminidase enzyme test in leukocytes/fibroblasts (13) and AGA mutation analysis (12)

Sample 2013-2G: Lysinuric Protein Intolerance (LPI)

Clinical description: A 4 year old boy with splenomegaly (known since 6 months of life), failure to thrive and a special eating behavior. The sample was collected at the age of 17 years during a routine checkup while receiving specific treatment.

Sample G was the common sample and was provided by DPT Czech Republic. Results were discussed by dr Victor Kozich during the ERNDIM Workshop in Barcelona on September 3, 2013. The presentation is available at the ERNDIM.ORG website.
The common sample was from a patient suffering from Lysinuric Protein Intolerance.



STAINING:	a.Ninhydrine	b.Orcinol
5. GlcNAc-Asn	positive	
4. (GalGlcNAc)-Asn	positive	positive
3. abnormale band		positive
2. abnormale band		positive
1. abnormale band		positive

Fig. 6. Oligosaccharide TLC patterns of aspartylglucosaminuria urine with A: ninhydrin staining and B: orcinol staining (kindly provided by dr Kluijtmans and dr Huigen, Radboud University Medical Centre, Nijmegen).

6 Preview of the 2014 scheme

The format of the DPT scheme in 2014 will be identical to previous years. The logistics of the scheme will change, however. In order to achieve European harmonization, all samples will be shipped from a central point, i.e. the CSCQ headquarters in Geneva. For the same reason all six samples will be dispatched in one shipment and participants will be asked to analyse the samples in two pairs of three. For reporting, the CSCQ website will only be open in the corresponding time slots, which will be 3 weeks.
Results will be discussed during the ERNDIM workshop just before the SSIEM symposium, which will be in Innsbruck, Austria in 2014.

Starting with the 2014 schemes the concept of 'critical error' will be introduced to the assessment of the DPT schemes. A critical error is an error that would be unacceptable to the majority of labs and would have a serious adverse effect on patient management. The introduction of critical error is on the advice of the Genetic Services Quality Committee (GSQC) of the European Society of Human Genetics (ESHG), which wants to see harmonisation across all European genetic EQA providers. A confirmed critical error will mean automatic classification as a poor performer. The final scoring of all qualitative schemes will be discussed at the Spring meeting of the Scientific Advisory Board (SAB) and all proposed critical errors will need to be ratified by the SAB before being confirmed.

In order to explain the concept of critical error, a short description of possible critical errors in the 2013 samples is described below (reminder: critical error will not be applied for the 2013 samples). Please note that criteria to establish critical error are context-dependent and hence criteria used for a particular sample in one survey may be different compared to a similar sample used in another survey.

Sample 2013-1A	Failure to detect elevated mma in organic acid analysis and any diagnosis other than MMA would be classified as a critical error.
Sample 2013-1B	The relatively low proficiency of this sample shows that MNGIE is not a straightforward diagnosis to establish. Critical error is not applicable for this sample.
Sample 2013-1C	Educational sample (CTX) ; no critical error.

Sample 2013-2D	Failure to detect elevated ornithine in amino acid analysis and failure to conclude OAT deficiency (gyrate atrophy) would be classified as a critical error.
Sample 2013-2E	Aspartylglucosaminuria is currently not subject to critical errors.
Sample 2013-2G	Failure to detect both orotic acid and elevations of lys, arg, orn would be classified as a critical error. Also failure to establish the diagnosis LPI on the basis of (partially) abnormal analytical findings is a critical error.

7 Minutes of the ERNDIM DPT NL 2013 discussion

Barcelona, September 3, 2013, 8.30-10.30

Attendants: M Dercksen (Potchefstroom), J Bierau (Maastricht), I Körver-Keularts (Maastricht), L Greed (Perth), J Dewulf (Brussels), M Wamelink (Amsterdam), P Burda (Zurich), J Sass (Zurich), W Onkenhout (Leiden), M de Sain (Utrecht), B Prinsen (Utrecht), L Kluijtmans (Nijmegen), U Engelke (Nijmegen). B Jacobs (Tilburg), F Boemer (Liege).

1. Minutes of the meeting in Birmingham on September 4, 2012 were approved

2. News from ERNDIM
- SSIEM Academy 2014 will take place on April 1-2 in Paris. The topics will be Lysosomal, peroxisomal and purine/pyrimidine disorders.
- In collaboration with SKML, ERNDIM has developed a oligosaccharide kit: a collection of positive urine samples. The kit is available through SKML. Samples of patients with alpha-fucosidosis and beta-mannosidosis are still lacking. Participants are kindly requested to send in urine samples (50 ml minimum) of these patients.

3. Website reporting
- Website reporting has been used successfully in 2013, although some problems recur, such as premature closure of the site.
- Comments and suggestions can be sent to George Ruijter or Xavier Albe (CSCQ, Xavier.Albe@hcuge.ch). A manual of the website will be provided by X. Albe on request.

4. Planning and organization of DPT Netherlands in 2014
- Organization of DPT NL will be done by CSCQ in 2014. All six samples will be shipped in one package. The moment of shipment depends on availability of the common sample and may be later than the usual start (early February) of survey 1. Starting and closing dates of the 2 surveys will be communicated by email. At the starting date of a survey the website will be opened to retrieve the clinical information and to submit results.

5. Any other business
- Unfortunately a sample mix-up has occurred in DPT NL survey 2013-2. Instead of the common sample provided by DPT CZ (Victor Kozich) another sample has been circulated. The mistake has occurred in the scheme organizer's lab (MCA lab, Winterswijk) and could be tracked to misunderstandings between Scheme Organizer and Scientific Advisor. We are taking this matter very seriously and sincerely apologize. The required corrective action has been carried out: the correct sample has been sent to the participants. Measures to prevent this in the future have been developed. A summary of the results reported for the 'erroneous' sample (F) has been circulated.

- Finally, all 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.

Rotterdam, March 21, 2014

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