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ANNUAL REPORT 2011

1. Introduction

The Diagnostic Proficiency Test (DPT) scheme for inborn errors of metabolism is run by ERNDIM as before and continues to be the ultimate challenge for diagnostic labs. The Wintyerswijk/Amsterdam/Rotterdam scheme historically has participants from the Benelux region as well as from the North-western part of Germany and currently includes one participant from South Africa.
Following the previous announcement of website reporting of the DPT-findings, the system has been put into action during the year 2011. Accordingly the reports of the second set of samples in 2011 were submitted electronically on the website (https://baal.hcuge.ch/cscq/ERNDIM/Initial/Initial.php). In line with previous discussions the time allotted for submitting reports was extended to 4 weeks with the date of shipping the samples as starting point.
Since 20 years this scheme has been run in conjunction with SKML, the Dutch QA-organization for medical laboratories. The handling of samples has been transferred to the Winterswijk location in 2010. Accordingly, dr.C.Weykamp has been appointed as scheme organizer. In order to achieve a more uniform scoring panel throughout the five DPT schemes, the ERNIM Board has instituted a slight change in the rules of scoring by adding a second scoring officer from one of the partner DPT schemes. The external scores will be discussed with the scheme's own scientific advisor(s). In case of the Winterwijk/Amsterdam/Rotterdam scheme, additional scores will be made by the scientific advisor of the Basel scheme.

This year's scheme consisted of six urine samples, distributed in January and June; the discussion of the results took place in Geneva on the occasion of the ERNDIM workshop held at the SSIEM Annual Symposium on August 30. The meeting, as usual open to participants only, was attended by representatives of most of the participating institutes. George Ruijter, Erasmus Medical Center Rotterdam, chaired the meeting and prepared the summary of the discussions, which is part of the annual report.

2 Participants

The 2011 scheme had 19 participating laboratories with the following allocations:

Country	Number of participants
Luxembourg	1
Belgium	5
The Netherlands	11
Germany	1
South-Africa	1

3 Logistics of the scheme

Shipment of samples was effected as in previous years by regular mail. As discussed before this may cause some delay for remote laboratories. This was exemplified by the South-African participants, who found out that they had only one week for their analyses. This potential drawback has been overcome by sending the samples to the South African participants two weeks prior to the regular shipment.
The pan-European sample was supplied by us. Its results were discussed at the 2011 Annual Symposium of SSIEM as part of the ERNDIM workshop. It regarded a patient with creatine deficiency as a result of guanidinoacetate methyltransferase deficiency. During the ERNDIM workshop a considerable discussion on the appropriateness of the sample was raised, mainly because it was not entirely clear whether a biochemical genetics laboratory would be expected to offer an analysis of creatine and guanidinoacetate. In the light of the progress of diagnostic developments as well as the promising treatment options of the creatine biosynthesis defects, the scientific advisors of the present scheme are very much in favour of implementing the analysis of creatine and guanidinoacetate in every biochemical genetics laboratory.

4 Scoring of results

For each individual sample a score can be achieved for:		
		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)	2
	Helpful but incomplete	1
	Misleading / wrong diagnosis	0
Recommendations: (for further investigations)	Helpful	1
	Unsatisfactory or misleading	0
	Total score	5

The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. During its meeting in Manchester on November 4, 2011, the Board decided that it was not appropriate to score the results on the 2011 common sample. In accordance with this decision, participants who failed to achieve satisfactory performance were those who scored less than 15 points out of the maximum 25 in this year. These so-called poor performers have received a performance advice letter from the Scientific Advisors which is explicitly aimed at improving the participant's performance in the near future.

5 **Results of individual samples**

Performance on the 6 samples was as follows

Sample	Diagnosis	No. of reports	Correct diagnosis (%)	Partially correct diagnosis (%)
A	L-2-OH-glutaric aciduria	18	18 (100)	-
B	Mild homocystinuria	18	2 (11)	0
C	Mucopolysaccharidosis type III	18	17 (95)	0
D	Molydenum cofactor deficiency	19	16 (84)	0
E	Argininosuccinate lyase deficiency	19	18 (95)	0
F	GAMT deficiency	19	12 (63)	1 (5)

The total number of reports was 111 out of the 114 which were expected on the basis of the number of registered participants. For all samples,83 out of 111 reports (75%) were correct, quite similar to the overall performance in 2010 and considerably better than in 2008 and 2009.

One of the samples scored quite badly. It came from a patient with presumed homocystinuria. Not only the clinical information was quite uninformative, but also the detection of the cysteine-homocysteine disulfide in the regularly performed amino acid analysis turned out to be troublesome. Several participants experienced that retrospective appreciation of their original amino acid analyzer results did reveal the presence of the mixed disulfide. In this respect the educational value of the DPT scheme has once more been highlighted. Unfortunately the sample did not contain homocystine, thereby complicating the final diagnosis.

6. Minutes of the ERNDIM DPT Amsterdam 2011 discussion

Geneva, August 30 2011, 9.00-10.30

1. Minutes of the meeting in Istanbul on August 31, 2010 were approved

2. News from ERNDIM

- Accreditation of ERNDIM (mainly the office): regular meetings with the EMQN, the molecular genetics network, are ongoing. ERNDIM/SSIEM have appointed Sarah Gardner as a scientific administrator in February 2011. She is based in the Manchester office of EMQN and will prepare ERNDIM accreditation and assist ETAC
- DPT results will be assessed by two independent scientific advisors. DPT Amsterdam will also be judged by Prof. Brian Fowler.
- ERNDIM is considering a neurotransmitter pilot scheme for the diagnostic approach of neurometabolic disease in cerebrospinal fluid, including the analysis of neurotransmitter metabolites. This is a long-standing wish of many of the participants..
- ETAC training will take place on November 23/24 in Amsterdam. November 25 will be the retirement symposium of Cornelis Jakobs. The ETAC training will have a format similar to 2010 and includes 3 topics over 2 days: GSDs, CDG syndromes and mitochondrial disease.

3. Website reporting

Website reporting has been implemented in 2011. DPT Amsterdam started with survey 2011-2, the second set of samples. Accounts and passwords have been circulated again to participants in June. The reporting deadline has been postponed by a week from july 4 to july 11, 2011 to accommodate participants experiencing problems with method/data entry. Comments and suggestions can be sent to George Ruijter.

4. Any other business

a. 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.
b. Please indicate in your report which analyses have been performed by a clusterlab. Proficiency/performance testing includes establishing whether labs have a complete panel of analyses.
c. ERNDIM strongly advises to be specific in the report section 'recommendations'. Recommendations are context sensitive and sometimes difficult to assess. Therefore, scoring recommendations is currently quite relaxed, but we will move to more strict scoring of this item. For example, an non-specific statement such as 'perform enzyme testing and mutation analysis' is currently scored 1 point. In the future it will be required to include specific enzyme and gene names in this particular example.

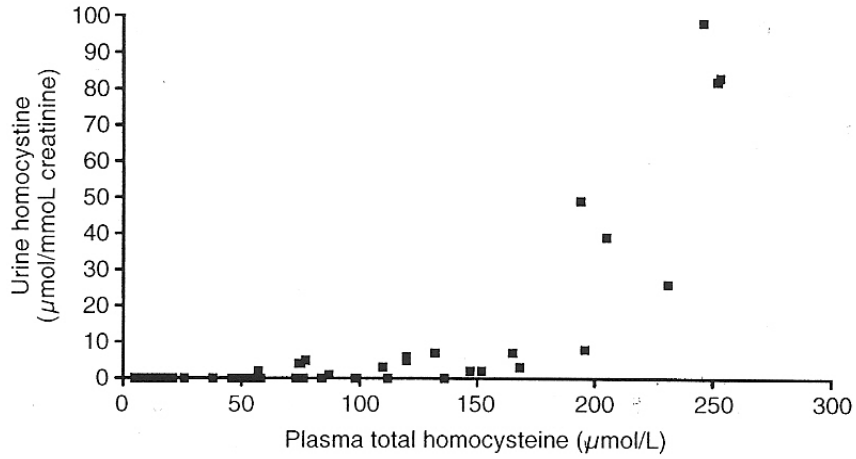
Discussion of the 2011 samples A-B-C-D-E (F was the common sample).

A. L-2-OH-glutaric aciduria

Clinical description: A 35-year-old mentally retarded female with progressive dementia and epilepsy. MRI of the brain showed a leukodystrophy. She is being treated with valproate.
Target values were: Analytical: elevated (L) 2-HG (optional: normal EMA / dicarboxylic acids / glutaric acid to distinguish from MADD), Interpretation: (L) 2-OH-glutaric aciduria
DD leukodystrophy includes: MLD, Krabbe, L-2-OH-glutaric aciduria, respiratory chain disorders, GM1, GM2, X-ALD, the latter less likely in a female patient.
2-HG averaged 1363 mmol/mol creat (range 217-3920), while L- 2-HG determination was specifically mentioned by 3 labs. 3-HIVA was reported to be elevated due to valproate by 9 labs.
Recommendations for further investigations included: D/L separation, mutation analysis of L2HGDH (duranin), MRI, enzyme analysis and determination of CSF lysine.

B. Mild homocysteinuria/hyperhomocysteinemia

Clinical description: Patient is a 54-year-old male who has progressive ataxia, predominantly of the lower extremities, since about 7 years. Diagnostic imaging showed cerebellar atrophy.
Target values were:
This was a particularly challenging sample. Two labs diagnosed hyperhomocysteinuria/ hyperhomocysteinemia, while 12 labs reported 'No diagnosis'. The sample contained slightly elevated cys-hcys mixed disulfide (elutes just before tyrosine in Biochrom amino acid analyzer), but normal homocystine and MMA. One laboratory reported a positive Brand reaction (cyanide-nitroprusside test), which is a qualitative test to determine sulfhydryl compounds .
Seven labs reported the presence of a large quantity 2-ethoxyethoxyacetate. This compound has been described in the literature as a carbitol metabolite (J.P.Kamerling et al, 1977, Clin Chim Acta 77:397-405).
In plasma of this patient total homocysteine was 97 µmol/L. According to data reported by Moat et al (1999 Ann Clin Biochem 36:372-379) this barely results in elevated urine homocystine (see figure).



C. Mucopolysaccharidosis type III
Clinical description: A female aged 26 years who presented with early dementia and loss of function.
Target values were: Analytical: elevated GAG + heparansulfaturia, Interpretation: MPS III.
Reported values for GAG were on average 21 mg/mmol creat (range 13.9 – 28). Diagnostic proficiency was excellent for this sample of a mild MPS IIIA patient: 95 %. Previous MPS III samples were diagnosed less well: sample 2008-A: mild MPS III B, 6 x correct diagnosis, 2 x partially correct diagnosis; sample 2008-F: MPS IIIA with an non-specific clinical picture, 9 x correct diagnosis, 5 x partially correct diagnosis. It can be hypothesized that participation in the recently introduced mucopolysaccharide DPT-scheme results in improvement of the performance in the MPS-samples of the general DPT-schemes.

D. Molydenum cofactor deficiency
Clinical description: This boy was born as the first child of a consanguineous couple. He was referred at the age of 2½ years because of severe mental retardation, hypertonicity and recurring infections.
Target values were: Analytical: decreased uric acid + elevated xanthine + hypoxanthine + elevated S-sulfocysteine (optional: elevated taurine), Interpretation: Molybdenum cofactor deficiency.
Reported sulfocysteine values were on average 201 mmol/mol creat (range 116-285), while taurine was also elevated: 402 mmol/mol creat (314-489). Samples with elevated taurine must be checked for sulfocysteine.
Values for the purine metabolites were: uric acid, average 2.5 mmol/mol creat (range 0-13); xanthine, 122 (100-154) and hypoxanthine, 67 (46-99)
Sulphite was reported as positive by 5 labs and negative by 7 labs. Two labs found a trace of sulphite. In this pooled urine sample which had been stored at -20° for 19 years, sulphite was originally + to +++ in the different samples, which corroborates that sulphite disappears slowly during storage/freezing..
Several participants mentioned the presence of glyceric acid, but the reported values were lower than observed for Hyperoxaluria type 2 or D-glycerate kinase deficiency.
Recommendation for further investigations included: thiosulfate determination, enzyme analysis (liver biopsy required !), complementation analysis, mutation analysis (MOCS1, MOCS2, GPHN).
Advice to the attending clinician included: cPMP treatment and a low protein/sulfur diet (only effective in mild cases).

E. Argininosuccinate lyase deficiency
Clinical description: This female patient was referred at the age of 5 years because of ataxia. It was noticed that she had brittle hair. The urine sample was obtained at the age of 28 years.
Target values were: Analytical, elevated argininosuccinate (optional: the presence of cyclic metabolites or anhydrides; Interpretation: Argininosuccinate lyase deficiency/Argininosuccinic aciduria.
The DD brittle hair is rather limited and includes: ASL deficiency, Trichothiodystrophy (TTD), Menkes syndrome.
This sample was nitrite-positive, but this was not considered to be a problem.
Argininosuccinate values averaged 1522 mmol/mol (range 213-3800). Presumably the range of reported values is extended due to variable anhydride formation on column.
Only traces of orotic acid were observed and most participants reported orotic acid normal.
Uracil was reported as elevated by 8 labs. The origin of uracil in this sample is most probably bacterial degradation of pseudo-uridine.
Advice for further investigations included: plasma amino acid analysis, enzyme assay in RBC/fib, mutation analysis.
Advice to the attending clinician included: a low protein diet, Arg supplementation, Benzoate/phenylbutyrate administration, to monitor liver function, liver transplant