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ANNUAL REPORT 2012

1 Introduction

The Diagnostic Proficiency Test (DPT) schemes for inborn errors of metabolism are run by ERNDIM and continue to be the ultimate challenge for biochemical genetic labs. The Amsterdam-Winterswijk-Rotterdam (AWR) scheme historically has participants from the Benelux region as well as from the North-western part of Germany and currently includes participants from South Africa and Australia.
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 organizer.

The minimal required test panel for for participation in any DPT scheme includes dip stick, amino acids, organic acids and quantitative GAG. DPT AWR additionally requires the analysis of oligosaccharides and purines-pyrimidines.
It is strongly recommended to have the following tests available for DPT AWR: 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 other 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.

The reports of the samples in 2012 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 4 weeks with the date of shipping the samples as starting point.
In order to achieve harmonised scoring throughout the five DPT schemes, the ERNDIM Board has instituted a slight change in the rules of scoring by adding a second scoring officer from one of the partner DPT schemes as of 2011. The external scores will be discussed with the scheme's own scientific advisor(s). In case of the AWR scheme, additional scores have been made by the scientific advisor of the Basel scheme.

This year's scheme consisted of six urine samples, distributed in two surveys: 3 samples in February and 3 samples in June; the discussion of the results took place in Birmingham during the ERNDIM workshop held at the SSIEM Annual Symposium on September 4, 2012. 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 2012 scheme had 18 participating laboratories with the following allocations

Country	Number of participants
Australia	1
Belgium	5
Germany	1
The Netherlands	10
South-Africa	1

3 Logistics of the scheme

Samples were dispatched, as in previous years, by regular mail. As discussed before, this may cause some delay for laboratories outside Europe. This was exemplified by the South-African participants, who found out that they had only one week left for their analyses. This potential drawback has been overcome by sending the samples to the Australian and South African participants two weeks prior to the regular shipment.
The pan-European sample was provided by DPT Sheffield. Results were discussed by dr.J.Bonham at the ERNDIM workshop as part of the 2012 Annual Symposium of SSIEM in Birmingham. The common sample was from a patient with a mild form of MSUD.

4 Scoring of results

Criteria for scoring results are as follows:

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
	Absent, unsatisfactory or misleading	0
	Total maximal score	5

The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. During its meeting in London on November 29, 2012, the Board decided that for the common sample (sample F; MSUD) the analytical performance would be scored as follows: 1 point for reporting elevated leucine/isoleucine/allo-isoleucine, and 1 point for reporting at least one 2-oxo-acid/2-hydroxyacid characteristic of MSUD (e.g. 2-OH-isovaleric acid).
All samples of DPT AWR were scored (none of the samples were removed from scoring) and in accordance with a previous decision by the board, participants who failed to achieve satisfactory performance were those who scored less than 18 points out of the maximum of 30 in this year.

5 Results of individual samples

Performance on the 6 samples was as follows

Sample	Diagnosis	No. of reports	Correct diagnosis (%)	Partially correct diagnosis (%)
A	Isovaleric acidemia	18	18 (100)	-
B	3-Methyl-3-Hydroxybutyryl-CoA dehydrogenase deficiency	18	7 (39)	-
C	Galactosemia	18	18 (100)	-
D	Adenylosuccinase deficiency	18	11 (61)	-
E	Alfa mannosidosis	18	15 (83)	-
F	MSUD	18	15 (83)	-

The total number of reports was 108 out of the 108 which were expected on the basis of the number of registered participants. For all samples, 84 out of 108 reports (78%) were correct, slightly better than the overall performance in 2011 (75%) and 2010 (77%). Two samples (A, IVA and C, galactosemia) were diagnosed correctly by all participants. Sample B (2-methyl-3-OH-butyryl-CoA dehydrogenase deficiency) was challenging as only 7 laboratories reported the correct diagnosis (see also discussion of individual samples below; item 7.5).

6 Preview of the 2013 scheme

The format of the DPT scheme in 2013 will be identical to previous years. The scoring of results will, however, change in 2013.

Scoring of recommendations
At a special meeting of the Scientific Advisory Board in November it was agreed that from 2013 onwards the qualitative schemes will all adopt the same scoring system of two points for analytical results and two points for interpretation. For the DPT scheme, there will no longer be an additional point for suggested further actions or testing. This will be included as part of the interpretation. This will also reduce the total score achievable to twenty four (with six samples) and acceptable performance will be achieved by scoring at least fourteen points.

Critical Error

The Scientific Advisory Board is now discussing the definition of 'critical error'. All participants will be informed on how this will affect the scoring. In principle this is a category of error that would be unacceptable to the majority of labs and would have a serious adverse effect on patient management. We are hoping to be able to institute this for 2014.

7 Minutes of the ERNDIM DPT Amsterdam-Winterswijk-Rotterdam 2012 discussion

Birmingham, September 4, 2012, 9.00-10.30

7.1. Minutes of the meeting in Geneva on August 30, 2011 were approved

7.2. News from ERNDIM

- ERNDIM has a new website and logo. In the future this website will be the portal to SKML (www.erndimqa.nl) and CSCQ (https://baal.hcuge.ch/cscq/ERNDIM/Initial/Initial.php) data entry websites
- SSIEM Academy 2012 will take place 2nd & 3rd October 2012 in Manchester, UK. The topic will be amino acids. In 2013 SSIEM Academy will be on the 16th and 17th of April in Lyon (organic acids incl. fatty acid oxidation).
- Currently ERNDIM in collaboration with SKML is developing an oligosaccharide kit: a collection of positive urine samples. Once the kit has been produced it will be available from SKML. Samples of patients with fucosidosis and beta-mannosidosis are still lacking. Participants who have such samples available are kindly requested to send in urine samples (50 ml minimum) of these patients.

7.3. Website reporting

- Website reporting has been used successfully in 2012, although some problems have occurred, such as slow performance. Evaluation software to be used by the scientific advisors is still under development.
- Comments on the software:
  - results of previous rounds are not available
  - the website often starts up in the French language
- George Ruijter will discuss these comments with the webmaster. Other comments and suggestions can be sent to George Ruijter.

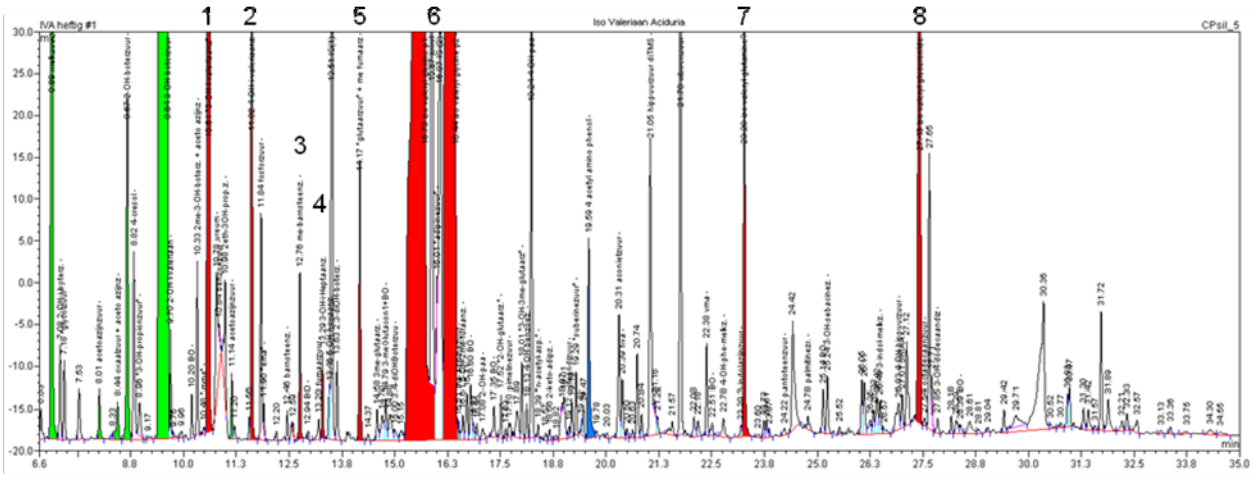
7.4. Any other business

- 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 indicate in your report which analyses have been performed by a clusterlab. We aim at including possibilities to indicate this on the website. Proficiency/performance testing includes establishing whether labs have a complete panel of analyses.
- Ben Poorthuis: do guidelines exist for writing result reports? George Ruijter: a document is available on the ERNDIM website with a description of writing a report. Official guidelines are not available probably because of large international differences.

7.5. Discussion of the 2012 samples A-B-C-D-E (F was the common sample).

A. Isovaleric acidemia
Clinical description:
The patient is an 8 day-old female. A few days after birth vomiting and feeding difficulties started. She was hypotonic with decreasing consciousness, leading to coma. Hyperammonemia or acidosis were not mentioned. The first child of the parents died during labour.
The following values were reported for the relevant metabolites. Isovalerylglycine: median 5173 mmol/mol (range 925-10000), 3-OH-isovaleric acid: median 422 mmol/mol (range 96-5765).

Chromatogram of a sample from a decompensated IVA patient (with peaks identified below):



1 3-HIVA (ω-1 hydroxylation)
2 4-HIVA (ω- hydroxylation)
3 Me-succinate (ω- oxidation)
4 3-OH-isoheptanoate (abnormal ketone, condensation of acetyl-CoA and isovaleryl-CoA)
5 Me-fumarate (mesaconate; ß-oxidation of 3)
6 Isovalerylglycine (two peaks with one and two TMS-groups resp.)
7 Isovalerylglutamate
8 Isovalerylglucuronide
All mass spectra are available on request

Isovalerylglycine is not part of the quantitative organic acids scheme. The labs present support the suggestion to include this component in the scheme.

B. 2-methyl-3-OH-butyryl-CoA dehydrogenase deficiency Clinical description:
A 4 year-old male with psychomotor retardation and dysmorphism. Medication is not known.

Many labs missed this diagnosis because the clinical description was not very specific and because of only slightly increased excretions of tiglylglycine and 2-methyl-3-hydroxybutyric acid. There were large interlaboratory differences in the quantitation of the two components. Notably, not all labs quantify organic acids at all, but in the case of this sample quantitation of the two abnormal metabolites would most certainly help to establish diagnosis. Tiglylglycine has been part of the ERNDIM quantitative organic acid scheme since its start. Reference values have been reported, 2-Me-3-OH-butyrate 11–27 mmol/mol creat; tiglylglycine < 3.8

(Poll-The et al. Mol Genet Metab. (2004) 81:295-299, this paper also describes the patient whose urine was used), but need to be established from own data.
The presence of tiglylglycine should trigger a suspicion of MHBD (in addition to propionic acidemia, oxothiolase deficiency). MHBD can be distinguished from oxothiolase deficiency on the basis of one form (peak) of 2-methyl-3-hydroxybutyric acid in MHBD and two forms (peaks) in oxthiolase deficiency as well as the absence/presence of 2-methyl-3-oxobutyric acid (2-methyl-acetoacetate). The latter compound is rather unstable and does not survive the pre-treatment that is applied for DPT-samples, as evidenced by previous DPT-results..
Tiglylglycine can be purchased from Herman ten Brink of VU Medical Centre.
MHBD is an example of a 'moonlighting' protein and is identical to the MRPP2 subunit of mitochondrial RNAse P, which is required for tRNA processing. The disease symptoms are currently believed to be mainly related to the RNAseP function.

C. Galactosemia
Clinical description:
A 9 day-old female with hyperbilirubinemia, vomiting, increased liver enzymes and weight reduction.
Most laboratories quantified galactose and/or galactitol. The 'reducing sugars' test is not performed on a routine basis anymore by many labs, since the Bayer Clinitest test tablets are our of stock. Although not certain, Clinitest may be available in the near future.
The clinical description was suggestive of galactosemia, but also of tyrosinemia type I and it is recommended to specifically exclude the latter diagnosis by inspecting the urine succinylacetone level. In fact, many participants did this.

D. Adenylosuccinase deficiency
Clinical description:
A 44 year old female with mental retardation and epilepsy for which she is treated with valproate. She was diagnosed at the age of 26.

The number of participants that came to the correct diagnosis for this sample was 11, which was a bit disappointing. Partly this is due to the fact that some participants do not perform purine analysis. In addition, relatively small amounts of succinyl-adenosine and succinyl-aminoimidazolecarboamide riboside (SAICAr) were present in sample D. The median SAICAr level was 16 mmol/mol creat(range 1.3-20). The wide range is caused by the lack of a calibrator. SAICAr is a very expensive component and can therefore not be included in the ERNDIM quantitative purine-pyrimidine scheme, but its analogue AICAR will be found in the Pur/Py scheme. Many laboratories have established a response factor for SAICAr based on the result of a positive sample in a previous DPT survey. The median level of succinyl-adenosine (s-Ado) was 50 mmol/mol (range 14-63). S-Ado is also not for sale, but can be prepared easily from adenylosuccinate (available from Sigma). A protocol is available on request. Reference values are age-dependent. Urine from young children usually does contain some s-Ado, which makes quantitative analysis imperative to identify ADSL patients. Two forms of ADSL deficiency are generally distinguished, i.e. a clinically severe form in which affected patients excrete roughly the same amounts of SAICAr and s-Ado and a somewhat attenuated form in which the urine s-Ado level is 3-4 times higher than that of SAICARr. Apparently the present patient has the attenuated form of the disease. ADSL activity can be analyzed in red blood cells, leukocytes and fibroblasts. Expression can be highly variable: ADSL is not necessarily deficient in all tissues.

E. Alfa-mannosidosis

Clinical description:

This boy was referred at the age of 4 years for psychomotor retardation, sleep disturbances, recurrent respiratory tract infections and a bulging abdomen. The urine sample was obtained at the age of 6 years.

The oligosaccharide pattern of an alfa-mannosidosis urine sample is very characteristic and many participants correctly diagnosed this patient.

Oligosaccharide profile of sample E