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1. Introduction

The Quality Assurance Program (QAP) for Diagnostic Proficiency Testing (DPT) of Inherited Metabolic Diseases is organised by ERNDIM. The existing DPT centres have encountered an increased demand on their services in 2005. Therefore it was decided to open a new DPT centre in Basel and reallocate some of the participants to the now five DPT centres, located in Sheffield, UK (North-western Europe); Prague, CZ (Eastern Europe), Nijmegen, NL (Central Europe), Lyon , F (South-western Europe) and Basel, CH (Middle and South-eastern Europe). Each centre continues to accommodate approx. 20 participants.

The SKML (previously called SKZL) is a Dutch QAP-organisation collecting samples of urine from patients with metabolic diseases (participants are obliged to deliver these samples). Twice a year SKML distributes these samples to the participants of the DPT scheme, evaluates all results, prepares a report and make the reports available to the participants. Once a year a meeting of the participants is organised to discuss the results, to bring faulty results into focus and to discuss recommendations for improvement. This meeting is chaired by the Scientific Advisor of the DPT scheme, currently dr. M. Duran, Academic Medical Center Amsterdam. Due to the fact that an International meeting on Inborn Errors of Metabolism was held far outside Europe, the Annual DPT meeting was incorporated in a two-day-meeting organized by ERNDIM and Eurogen test in Prague on 5-6th October, 2006.

2. Participants

In 2006 the DPT centre Central Europe had 19 participants from Belgium, Germany and The Netherlands.

Country	Number of participants
Belgium	2
Germany	7
The Netherlands	10

3. Logistics of the scheme

As in previous years, six urine samples were distributed in 2006 in two shipments. The samples were labelled A thru F. Sample F was the common sample, to be distributed by all five DPT centres. Reporting of the diagnostic results was done by e-mail in virtually all cases; fax reports appeared

incidentally and were accepted. The Scientific Advisory Board is currently discussing the possibilities of making a website-based reporting system, in analogy to the quantitative schemes.

4. Timetable of the scheme

DPT survey 2006.1:	
Shipment of samples:	09/01/2006
Deadline for returning results:	30/01/2006
Report of survey	15/07/2006
DPT survey 2006.2:	
Shipment of samples:	12/06/2006
Deadline for returning results:	03/07/2006
Report of survey:	06/09/2006

Annual meeting: October 5, 2006 Central Europe Centre, Prague, CZ. Seventeen representatives of the participating laboratories took part in the meeting.

5. Return of reports

Survey 2006.1: Reports on samples A, B and C were submitted by 19 participants
Survey 2006.2: Reports on samples D, E and F were submitted by 19 participants.

6. Scoring of samples and results

Following lengthy discussions in the Scientific Advisory Board, a scoring system was agreed upon in 2002.

For each individual sample a score can be achieved for:

Analytical performance:	Correct results of the appropriate tests	score: 2
	Partially correct or non-standard methods	score: 1
	Unsatisfactory or misleading	score: 0
Interpretative performance:	Good (diagnosis was established)	score: 2
	Helpful but incomplete	score: 1
	Misleading / wrong diagnosis	score: 0
Recommendations:	Helpful	score: 1
(for further investigations)	Unsatisfactory or misleading	score: 0
		=======
	Total:	score: 5

Poor performers are those participants who score less than 15 points out of the maximum 30 in a given year. These poor performers will receive a so-called warning letter from the Scientific Advisor.
At the request of several participants, individual scoring results will be sent to each individual participant.

7. Scores of participants for individual samples

Lab	A	B	C	D	E	F	Total 2006
1	5	5	5	5	5	1	26
2	5	5	5	5	5	5	30
3	5	5	5	5	5	5	30
4	5	2	5	5	4	5	26
5	5	5	5	5	5	5	30
6	0	1	5	5	4	0	15
7	5	1	5	5	5	5	26
8	0	5	5	5	5	5	25
9	5	5	5	5	5	5	30
10	0	5	5	5	5	5	25
11	5	1	5	5	5	3	24
12	5	5	5	5	5	5	30
13	0	1	5	5	5	5	21
14	0	2	0	5	0	0	7
15	5	2	5	5	5	5	27
16	5	1	5	5	5	5	26
17	0	1	5	0	0	0	6
18	0	1	5	5	5	1	17
19	0	1	5	5	5	5	21

8. Distribution of total scores 2005

		Number of labs
Better than 75%	(23-30 points)	13
Less than 75%	(<23%)	4
Less than 50%	(<15 points; poor performer)	2

9. Summary of scores

Sample	Diagnosis	Correct diagnosis made
A	Adenylosuccinase deficiency	11/19
B	Hartnup disease	8/19
C	Aspartoacylase deficiency (Canavan)	18/19
D	Methylmalonic acidemia	17/19
E	Hyperornithinemia (OAT)	15/19
F	Hypophosphatasia	13/19

The cumulative score for 2006 is 72% (82 correct diagnoses out of a maximum score of 114). This is slightly below the agreed level of 'good performance' set at 75%.

10. Minutes of the meeting for participants of the Diagnostic Proficiency Test (DPT) Centre of Metabolic Diseases for Central Europe held during the SSIEM Symposium in Prague, October 5, 2006

Present: 17 participants:
Amsterdam: Abeling, Duran (chairman) (AMC), Struijs, Wamelink (VUMC).
Bruxelles: Gerlo.
Groningen: Reijngoud.
Leiden: Onkenhout.
Liege: Boemer.
Maastricht: Bierau, Spaapen.
Nijmegen: Kluijtmans, Ruitenbeek (secretary).
Rotterdam: van Diggelen, Ruijter.
Tilburg: Jakobs.
Utrecht: Dorland, de Sain.

Welcome
The chairman welcomes the participants. The secretary of this DPT-scheme Sjoukje Holtrop has taken another job and will not longer attend the DPT meetings. A proposal is made to ask participants in turn to prepare minutes, which are important for future improvements.

Minutes of the meeting in Paris, September 6, 2005
Duran will check with Willems if the correction, mentioned under sample 2005.1 U, has been performed.
The minutes were accepted, with thanks to the secretary Nico Abeling.

Information from Executive Board, Trust Board, and Scientific Advisory Board
a) A fifth DPT centre has been established: Basel. Some participants had to be re-allocated.
b) There is a continuous need for new samples, suitable for the DPT analyses. 250 ml of urine is needed for one centre, 1000 ml for all 5 centres together.
c) The MPS centre in Manchester will stop its activities. Participants do not feel the need for another MPS scheme.
d) The advisory board will develop a website for the DPT and other proficiency test schemes. Viktor Kozich and Cas Weykamp are involved in the website development. The possibility of having a look into other DPT schemes (results, remarks etc.) will be discussed in the Advisory Board.
e) Participating laboratories will have 4 weeks for analysing and result reporting **starting from the moment that the above mentioned website is in the air.**
f) Several dates of report receipt are included in the recent summarizing reports of the SKML; some of them can not be correct.
g) The concentrations of the added compounds in the quantitative ERNDIM schemes must be either in the physiological or in the pathological ranges.
h) A certification form including the scoring results will be sent to every participating lab. The distribution of the scoring list of 2005 was very late.
i) A warning letter will be sent to laboratories with less than 50 % score in 2006.

Results of the 2006 survey
2006.1: samples A, B, C
2006.2: samples D, E, F.

Sample 2006.1-A: Adenylosuccinase Deficiency
High pH may point to bad conservation (Nijmegen will mention such findings to SKML). The use of valproic acid and vigabatrin has not been mentioned in the clinical history. The peak in the AAA in the area of OH-lysine has not been recognized by all labs as a vigabatrin peak. Six labs did not perform

analysis of purines/pyrimidines, five of them missed the diagnosis. Valproate induces increase of HIVA. Up to now SAICAR cannot be assayed Tandem MS. It was not clear whether a reliable enzyme assay is available, on the other hand, mutation screening is feasible.
11 of 19 labs found adenylosuccinase deficiency.

Sample 2006.1-B: Hartnup disease
The amino acid pattern looks at first sight that of generalized aminoaciduria, but in Hartnup proline excretion is normal and glycine excretion is relatively low. Indolacetic acid is produced in the gut from tryptophan, which is not resorbed. Oxo-proline (or pyroglutamic acid) is related to glutamine (degradation product).

Differentiation of γ-glutamyl cycle defects:

Defective enzyme	**Aminoaciduria**	**pyroglutamate**	**GSH**
γ-glucys synthase	+	-	Low
GSH synthase	-	+	Low
oxoprolinase	-	+	Normal
γ-GT	-	-	+ (urine)

Is analysis of amino acids in blood worthwhile in Hartnup disease? Probably not.
8 of 19 labs reported Hartnup as diagnosis.

Sample 2006.1-C: Canavan disease; accumulation of N-acetylaspartic acid.
DD of *nystagmus*, a symptom in this patient:
- 4-OH-butyric aciduria
- serine biosynthesis defects
- 3-Me-crotonylglycinuria
- 3-Me-glutaconic aciduria
- Caravan disease
- Cobalamin E, G, C
- MLD
- Salla disease
- Acrodermatitis enteropathica

No enzyme assay available as this gives quite variable results (communication by dr. O. van Diggelen).
18 of 19 labs found the correct diagnosis.

Sample 2006.2-D: Dialysis fluid of MMA patient.
High lactate concentration in dialysate is likely caused by the high lactate content in dialysis fluid. The composition of the dialysate has to be compared with blood rather than urine because no tubular reabsorption occurs during the dialysis process.
17 of 19 labs reported the diagnosis MMA.

Sample 2006.2-E: hyperornithinemia due to OAT deficiency (gyrate atrophy).
Remarkably small variation in ornithine concentration has been found. 3-amino-piperidone is often present in OAT deficient patients. Its location likely depends on equipment and method (at 3-Me-histidine location?; after arginine?); not mentioned by most labs. Guanidinoacetate should be decreased in these patients.
15 of 19 labs reported the correct diagnosis.

Sample 2006.2-E: hypophosphatasia (plenary discussion; common sample for all 5 centres).
Premature shedding of primary teeth is on important clue. Large variation in clinical spectrum and phosphoethanolamine excretion. See: Herasse et al (2004) J Med Genet 49, 605-609.

Actual situation: PEA can not be determined by Tandem MS.
13 of 19 labs reported hypophosphatasia as diagnosis.

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Prague, October 5, 2006
W. Ruitenbeek