ERNDIM DPT Center Prague

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Proficiency Testing Centre Prague
Annual Report 2012

1. Introduction

In 2011 proficiency testing in our centre was running as a regular ERNDIM scheme.

2. Geographical distribution of participants

Seventeen laboratories from 12 countries have participated in our Diagnostic Proficiency Testing scheme in 2012, for details see the below table:

Country	Number of participants
Croatia	1
Cyprus	1
Czech Republic	1
Denmark	1
Finland	1
France	1
Germany	4
Greece	1
Latvia	1
Malaysia	1
Poland	1
Slovakia	3
in total	**17**

3. Logistics of the scheme

- ✓ Two surveys: 2012/1 – samples A, B and C
  2012/2 – samples D, E and F

  Origin of samples: Five urines obtained from patients with known diagnoses (samples were provided by the DPTC participants and by the organizers) + a common sample from DPTC Sheffield (distributed in all five DPT schemes).

  The samples with the exception of the common sample F have been reanalyzed in our lab after heat-treatment. The diagnostically relevant metabolites were detected in all five samples after 3-day incubation at RT.
- ✓ Six heat-treated urines together with results protocols were distributed to the participants at ambient temperature using the courier FedEx. Based on the report of the courier 16 parcels were delivered within 3 days; we consider this transportation time acceptable.
- ✓ The following protocol for heat inactivation is being used: Thiomersal 100 mg/l of urine is added and urine is heated at 56 °C for one hour in water bath (this temperature is checked in urinary sample and not only in the water bath). The urinary samples have been frozen until shipment.

- ✓ Tests required in 2012: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2012

Sample distribution	March 26, Monday
Start of analysis of Survey 2012/1	April 16, Monday
Survey 2012/1 – results submission	May 11, Friday
Survey 2012/1 – report	June 1, Friday
Start of analysis of Survey 2012/2	June 11, Monday
Survey 2012/2 – results submission	June 29, Friday
Survey 2012/2 – report	August 10, Friday
Annual meeting of participants	September 4, Tuesday
Annual report 2012	December 16, Monday

5. The receipt of samples and results

Date of receipt of samples (samples sent on March 26, 2012)

date of receipt (reported by participants)	number of participants	date of receipt (reported by courier service)	number of participants
1 day	7	1 days	10
2 days	5	2 days	6
3 days	2	4 days	1
8 days	1	-	-
not indicated	0	-	-

Submission of results

	2012/1	2012/2
in time	17	17

6. Samples

Sample A

The sample was obtained from a 13 months old boy suffering from homocystinuria due to cystathionine beta-synthase deficiency. The diagnosis was established by enzyme analysis and completed by molecular analysis. The sample was taken from our repository while patient received dietary treatment, pyridoxine and betaine.

Analytical performance: All participants analyzed amino acids and 12 of them reported high excretion of homocystine, these results were considered correct. The analytical performance was suboptimal (71) %.

Interpretative proficiency: The diagnosis of homocystinuria and/or CBS deficiency was considered correct while suspicion for remethylation types of homocystinuria was considered helpful but incomplete. The interpretative proficiency score for this sample was poor (65%). Mostly due to overinterpretation of analytical findings. In our opinion urinary analysis of amino acids does not permit reliable differentiation between transulfuration and remethylation types of homocystinuria.

Recommendations: In our opinion the most important advice for follow-up investigation included the following recommendations: a/ amino acids (methionine) and/or homocysteine in plasma and b/ CBS activity measurement and/or mutation analysis. Both recommendations a/ and b/ were considered complete.

Overall impression: Typical DPT sample with suboptimal proficiency score.

Sample B

Patient: This sample came from a 3 months old boy with mevalonic aciduria due to mevalonate kinase deficiency. The diagnosis was established by enzyme analysis and completed by molecular analysis. This sample was contributed by Dr. Wanda Gradowska from the Children´s Memorial Health Institute in Warsaw.

Analytical performance: Elevated excretion of mevalonolactone was considered correct analytical results. All labs analyzed organic acid and all reported elevated concentration of mevalonolactone. The analytical performance of this sample was excellent (100%).

Interpretative proficiency: The diagnosis of mevalonic aciduria due mevalonate kinase deficiency was considered correct. The proficiency score for this sample was excellent (100%).

Recommendations: Confirmation of diagnosis by enzyme assay of mevalonate kinase activity in fibroblasts or lymphocytes and/or mutation analysis was considered helpful.

Overall impression: Typical DPT sample with good proficiency score.

Sample C

Patient: This sample came from a 8 years old boy with iminodipeptiduria due to prolidase deficiency. The diagnosis is solely based on demonstrating the urinary excretion of iminodipeptids. This sample was contributed by Dr. Wanda Gradowska from the Children´s Memorial Health Institute in Warsaw.

Analytical performance: The presence of iminodipetides in amino acids analysis of native urine and/or elevated concentration of proline and hydroxyproline after acidic hydrolysis of urine were considered a correct result. All labs have done analysis of amino acid, but only seven reported elevated excretion of iminodipetides. The analytical performance was rather poor reaching only 44%.

Interpretative proficiency: Prolidase deficiency was considered the correct diagnosis. The interpretative proficiency score for this sample in laboratories that detected increased excretion of iminodipeptids was good. The proficiency score of 47% was below the usual performance of our group.

Recommendations: Confirmation of diagnosis by enzyme assay of prolidase activity in fibroblasts or erythrocytes or lymphocytes and/or mutation analysis was considered helpful.

Overall impression: The total proficiency score (48%) of this moderately difficult DPT sample from a patient with a rare IEM was rather poor.

Sample D

The sample was obtained from a 7 years old boy suffering from mucopolysaccharidosis type I due to deficiency of α-L-iduronidase. The diagnosis was confirmed by enzymatic analysis. This sample was contributed by the Dr. Darina Behulova from Department of Clinical Biochemistry of University Children's Hospital in Bratislava.

Analytical performance: Elevated excretion of glycosaminoglycans and increased proportion of dermatan sulphate were considered a correct analytical result. Increased excretion of GAGs without report on dermatan sulphate elevation was scored as partially correct. Analytical performance was good (85) %.

Interpretative proficiency: The diagnosis of mucopolysaccharidosis type I was considered correct while suspicion for MPS (other types of MPS or non-specified MPS) was considered helpful but incomplete. The interpretative proficiency score for this sample was good (88%).

Recommendations: As this sample does not permit unequivocal diagnostic conclusion the organizers scored the recommendations in the context of analytical methods used by the laboratory. For participants who evaluated GAG fractions the measurement of appropriate enzymes (α-L-iduronidase, N-acetylgalactosamine-4-sulphatase, iduronate-2-sulphatase in leucocytes/fibroblasts) in leukocytes or cultured fibroblasts was considered helpful. Recommendation to carry out analysis of GAG for those participants that did not perform GAG analysis was considered also helpful.

Overall impression: Typical DPT sample with good proficiency score.

Sample E

Patient: This sample came from a 8 years old boy with Barth syndrome (3-methylglutaconic aciduria type II) due to mutation in tafazzin gene. The diagnosis was confirmed by molecular genetic analysis. This sample was contributed by Dr. Wanda Gradowska from the Children´s Memorial Health Institute in Warsaw.

Analytical performance: Elevated excretion of 3-methylglutaconate and/or 3-methylglutarate was considered correct analytical results. The analytical performance was good (82%), only 3 labs failed to detect 3-methylglutaconate.

Interpretative proficiency: The diagnosis of Barth syndrome (3-methylglutaconic aciduria type II) was considered correct while suspicion for other types of 3-methylglutaconic aciduria was considered helpful but incomplete. The proficiency score for this sample was slightly suboptimal (79%).

Recommendations: Confirmation of diagnosis by mutation analysis was considered helpful.

Overall impression: Typical DPT sample with slightly suboptimal proficiency score.

Sample F (common sample)

Patient: The common sample provided by the DPTC Sheffield was obtained from a 6-year old boy with intermittent form of maple syrup urine disease.

Analytical performance: All participants performed analysis of organic acids and amino acids. 16 participants observed the increased excretion of branched-chain 2-keto and 2-hydroxyacids, such analytical finding was considered correct and scored by 1 point. 12 participants detected mildly elevated excretion of branched-chain amino acids, such analytical finding was also considered correct and scored by 1 point. The analytical performance for this sample in organic acids analysis was good (82%).

Interpretative proficiency: Maple syrup urine disease was considered correct diagnosis. 15 labs concluded the correct diagnosis and thus the interpretative proficiency for this sample was good (88%).

Recommendations: Confirmation of diagnosis by amino acids analysis in serum and/or enzyme assay of branched-chain keto acid dehydrogenase complex activity in fibroblasts or lymphocytes and/or mutation analysis of *BCKDHA, BCKDHB, DBT* was considered helpful.

Overall impression: The total proficiency score (87%) of this easy DPT sample was good.

7. Scoring of results

Three criteria have being evaluated: analytical performance, interpretative proficiency and recommendations for further investigations. Due to the large variability in reporting results in various countries recommendations to treatment are not evaluated in proficiency testing, however, they are still reported and summarized by the scheme organizers.

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory or misleading	0
I	Interpretative proficiency	Good (diagnosis was established)	2
		Helpful but incomplete	1
		Misleading/wrong diagnosis	0
R	Recommendations	Helpful	1
		Unsatisfactory or misleading	0

The total score was calculated as a sum of these three criteria. The maximum that can be achieved is 5 points per sample, i.e. 15 points per survey and 25 points in 2011 (i.e. excluding sample 2011F). There is a new procedure for scoring DPT Scheme; scores assigned by Prague organizer and agreed at the Annual Meeting have been reviewed by independent advisor from another DPT Centre and scoring is finalized after any possible discrepancies had been resolved at the autumn ERNDIM Scientific Advisory Board meeting.

8. Score of participants for individual samples

Survey 2012/1

Lab no	Sample A Homocystinuria				Sample B Mevalonic aciduria				Sample C Prolidase deficiency			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	2	2	1	**5**	0	0	1	**1**
2	0	1	1	**2**	2	2	1	**5**	2	2	1	**5**
3	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
4	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
5	2	1	0	**3**	2	2	1	**5**	0	0	0	**0**
6	0	0	0	**0**	2	2	1	**5**	0	0	0	**0**
7	2	1	1	**4**	2	2	1	**5**	2	2	1	**5**
8	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
9	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
10	2	2	0	**4**	2	2	0	**4**	2	2	1	**5**
11	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	0	0	0	**0**	2	2	1	**5**	0	0	1	**1**
14	2	1	1	**4**	2	2	1	**5**	0	0	0	**0**
15	2	2	0	**4**	2	2	1	**5**	0	0	0	**0**
16	0	0	1	**1**	2	2	1	**5**	1	2	1	**4**
17	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**

Survey 2012/2

Lab no	Sample D Mucopolysaccharidosis type I				Sample E Barth syndrome				Sample F Maple syrup urine disease			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
4	1	2	1	**4**	0	0	0	**0**	1	2	1	**4**
5	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
7	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
8	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
9	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
10	1	1	1	**3**	2	2	0	**4**	1	0	0	**1**
11	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	1	1	1	**3**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	2	1	0	**3**	1	2	1	**4**
15	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
16	0	0	1	**1**	2	2	1	**5**	2	2	1	**5**
17	2	2	1	**5**	0	0	0	**0**	0	0	1	**1**

A – Analytical score, I – Interpretative score, R – Recommendations, T – Total score

9. Total score of participants for individual surveys and their performance in 2012

Lab no	Survey 2012/1 [points]	Survey 2012/2 [points]	Total point 2012
1	11	15	**26**
2	12	15	**27**
3	10	15	**25**
4	10	8	**18**
5	8	14	**22**
6	5	15	**20**
7	14	15	**29**
8	10	10	**20**
9	15	15	**30**
10	13	8	**21**
11	10	15	**25**
12	15	15	**30**
13	6	13	**19**
14	9	12	**21**
15	9	15	**24**
16	10	11	**21**
17	15	6	**21**

10. Score summary in 2012

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
A	***Homocystinuria***	71	65	65	67
B	***Mevalonic aciduria***	100	100	94	99
C	***Prolidase deficiency***	44	47	59	48
D	***Mucopolysaccharidosis type I***	85	88	100	89
E	***Barth syndrome***	82	79	71	79
F	***Maple syrup urine disease***	82	88	94	87

"Easy" and "difficult" samples were included in the surveys. The analytical and interpretative performance was good to very good for most diagnoses.

11. Satisfactory performance

The participants who obtained 18 or more points within the calendar year are considered to be performing satisfactory. All seventeen laboratories achieved a satisfactory performance.

12. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Prague took place during the ERNDIM Meeting 2012 in Birmingham on 4th September 2012, eight laboratories were represented. The following items were discussed during the annual meeting of our DPT centre:

1. Information
    - ERNDIM is aiming at accrediting Schemes
    - changes in DPT (sample recruitment and distribution, web based system at CSCQ)

- SAB is developing a new concept (similar to other genetic disciplines) of the critical error for evaluation of performance; participants will be informed in advance about this changes

2. Tests required for to 2013
   - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Discussion of results of samples A-F
   - scoring of 2012 results proposed by Prague DPT center organizers has been subsequently evaluated by second reviewer from an independent center, discrepancies were resolved at November 2012 SAB meeting and final scoring is shown in the Annual report

13. Tentative schedule of DPT scheme and fee in 2013

Sample distribution	to be determined by the ERDNIM SAB
Start of analysis of Survey 2013/1	April 22, Monday
Survey 2013/1 – results submission	May 17, Friday
Survey 2013/1 – report	June 7, Friday
Start of analysis of Survey 2013/2	June 10, Monday
Survey 2013/2 – results submission	June 28, Friday
Survey 2013/2 – report	August 9, Friday
Annual meeting of participants	September 3, Tuesday
Annual report 2013	December 16, Monday

The annual meeting of participants will take place on September 3rd during the ICIEM Congress in Barcelona, Spain.

The Executive Board and Board of Trustees of ERNDIM determined the DPT fee for 2013 in the amount of 341 €.

14. Certificate of participation and performance in Proficiency Testing for 2012

Results of DPT Scheme are included in the Certificate of participation and performance, which are issued by ERNDIM.

Prague, December 16, 2012

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