ERNDIM DPT Center Prague

Institute of Inherited Metabolic Diseases
General Faculty Hospital
and
Charles University 1st Faculty of Medicine
Ke Karlovu 2, 128 08 Prague 2, Czech Republic
phone: ++420/224 967 161, 224 967 679
fax: ++420/224 967 081 or 224 967 119

Proficiency Testing Centre Prague
Annual Report 2008

1. Introduction

In 2008 proficiency testing in our centre was running as a regular ERNDIM scheme.

2. Geographical distribution of participants

Eighteen laboratories from 15 countries have participated in our Diagnostic Proficiency Testing scheme in 2008, for details see the below table:

Country	**Number of participants**
Austria	2
Croatia	1
Cyprus	1
Czech Republic	1
Denmark	1
Finland	1
France	1
Germany	2
Greece	1
Kingdom of Saudi Arabia	1
Latvia	1
Malaysia	1
Poland	1
Slovakia	2
Switzerland	1
in total	**18**

3. Logistics of the scheme

- ✓ Two surveys: 2008/1 – samples A, B and C
  2008/2 – samples D, E and F

Origin of samples: Six urines were obtained from patients with known diagnoses (samples were provided by the DPTC participants and by the organizers). The samples were re-analyzed in our Institute after 3 days incubation at ambient temperature (to mimic possible changes that might arise during transport). The crucial metabolites were detected in each sample.

- ✓ The organizers acknowledge Dr. Adolf Mühl, Dr. Darina Behulova and Dr. Miljenka Naradin for providing samples for 2008 surveys.

- ✓ 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 2008: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2008

Sample distribution	March 25, Tuesday
Start of analysis of Survey 2008/1	March 31, Monday
Survey 2008/1 – results submission	April 18, Friday
Survey 2008/1 – report	May 16, Friday
Start of analysis of Survey 2008/2	June 2, Monday
Survey 2008/2 – results submission	June 23, Monday
Survey 2008/2 – report	August 22, Friday
Annual meeting of participants	September 2, Tuesday
Annual report 2008	February 5, 2009, Thursday

5. The receipt of samples and results

Date of receipt of samples (samples sent on March 25, 2008)

date of receipt (reported by participants)	number of participants	date of receipt (reported by courier service)	number of participants
1 day	7	1 days	14
2 days	3	2 days	2
4 days	1	3 days	2
not indicated	7	-	-

Deadlines of the results submission

	2008/1	2008/2
in time	16	11
1 day delay	-	1
2 days delay	-	1
4 days delay	1	1
9 days delay (work overload)	-	1
16 days delay (reason not given)	-	1
no answer	1	1

6. Samples

Sample A

Patient: The sample was obtained from an 8-year old boy with succinic semialdehyde dehydrogenase deficiency. The diagnosis was established by enzyme analysis and completed by molecular analysis. This sample was contributed by the Dr. Miljenka Naradin from Clinical Institute of Laboratory Diagnosis in Zagreb.

Analytical performance: The presence of 4-hydroxybutyric aciduria was considered a correct analytical result. Although all labs have analyzed organic acids only 13 laboratories reported elevated concentration of 4-hydroxybutyric acid. One laboratory used GC/FID method, noted

increased excretion of 4,5-dihydroxyhexanoate and commented that 4-hydroxybutyric acid probably co-eluted with urea. This result was scored as partially correct. The analytical performance was average (79%), as 3 labs detected normal or almost normal pattern of OA.
Interpretative proficiency: Succinic semialdehyde dehydrogenase deficiency was considered correct diagnosis. The interpretative proficiency score for this sample in laboratories that detected 4-hydroxybutyrate or related metabolites was good, overall proficiency was average (82%).
Recommendations: Confirmation of diagnosis by enzymatic assay and/or mutation analysis was considered helpful.
Overall impression: Typical DPT sample with an average proficiency score.

Sample B
Patient: The urinary sample was obtained from a patient without any known inborn error of metabolism, the sample was provided by Dr. Marek Makara from the Department of Paediatric Neurology of the Motol University Hospital. This infant suffered from seizures, extensive metabolic screening including plasma and urinary amino acids, organic acids, purines and pyrimidines, galactitol and plasma carnitine did not reveal any specific abnormality. The sample was obtained when the patient was treated with valproate and levetiracetam.
Analytical performance: All participants performed analysis of organic acids and all detected metabolites of valproic acid, five labs also mentioned the presence of levetiracetam metabolites in urine. The major finding in this sample was the presence of valproate metabolites in urine, such analytical finding was considered correct and scored by 1 point. All participants reported increased excretion of glycine in urine, such analytical finding was also considered correct and scored by 1 point, nine laboratories proposed that hyperglycinuria might be secondary due to valproate treatment. The analytical performance for this sample was excellent (100%).
Interpretative proficiency: Scoring of diagnoses was quite difficult due to large variability of conclusions, we considered the report of "no IEM" or non-specific finding in conjunction with valproate treatment a good diagnosis, the diagnosis of non-ketotic hyperglycinemia was considered only partially correct. The interpretative proficiency for this sample was good (88%).
Recommendations: As the clinical picture and hyperglycinuria may have suggested the presence of non-ketotic hyperglycinemia in the infant, we scored the recommendation to exclude this diagnosis by measuring plasma and CSF glycine as correct.
Overall impression: Typical DPT sample with an average proficiency score.

Sample C
Patient: The sample was obtained from a 17-year old female with adenylosuccinate lyase deficiency (ADSL). Diagnosis in this patient was confirmed by enzymatic and molecular analyses. The sample was obtained from our repository.
Analytical performance: The presence of SAICAr and/or S-Ado was considered a correct analytical result regardless of the employed technique (TLC or HPLC). Due to its non-specifity positive Bratton-Marshall-test was scored as only partially correct. Eleven participants analyzed purines/pyrimidines profile, one participant was considering to carry out the P/P analysis, however, they were not able to submit the results because of technical problems in the cluster laboratory. It is surprising that of these eleven participants five labs did not detect either SAICAr or S-Ado and that only six participants detected them. Preanalytical degradation of the typical metabolites does not seem very likely as SAICAr and S-Ado were clearly present in heat treated samples even after 3 days incubation at room temperature. The analytical performance for this sample was poor (44%).
Interpretative proficiency: ADSL deficiency was considered correct diagnosis. In general, the interpretative proficiency for this sample was exceptionally poor owing to the low proportion of samples analyzed for SAICAr and/or P/P analysis and failure to detect the typical metabolites. Consequently the interpretative proficiency score for this sample was only 53%.
Recommendations: For those, who diagnosed ADSL correctly, the following recommendations were considered crucial – measurement of the ADSL activity and/or mutation analysis in ADSL

gene. For laboratories thatdid not identify ADSL deficiency and recommended P/P analysis, this recommendation was also considered sufficient as it would have the potential to establish the correct diagnosis.

Overall impression: A diagnostically difficult sample due to low SAICAr concentration with poor total proficiency score (54 %). It was discussed during the Annual Meeting that some laboratories failed in detecting SAICAr by TLC due to concentration below the detection limit. It is disturbing to note that the overall proficiency improved only marginally since the last circulation of a sample with this diagnosis in 2004 (overall proficiency was 28 %, five out of nine laboratories performing P/P analysis failed to detect SAICAr). It seems that P/P analysis continues to be a difficult and perhaps neglected area of laboratory diagnostics of IEM. It was recommended that the ADSL deficiency should be considered in each patient with unexplained psychomotor retardation and/or seizures and/or hypotonia; such clinical conditions are very common, therefore several screening tests for SAICAr detection were developed (e.g. Bratton-Marshall test reported by Laikind et al., Anal Biochem 156: 81-90, 1986 or TLC method reported by de Bree et al., Clin Chim Acta 156: 279-288, 1986). We strongly recommend considering introduction of these simple screening tests in all laboratories that do not have an easy access to profile analysis of P/P.

Sample D

Patient: The sample was obtained from an 8-year old boy with maple syrup urine disease. The diagnosis was originally based on urinary examination of organic acids, genetic analysis was requested but the result is not known. This sample was contributed by the Dr. Darina Behulova from Department of Clinical Biochemistry of University Children's Hospital in Bratislava.

Analytical performance: All 17 participants performed analysis of organic acids and observed the increased excretion of branched-chain 2-keto and 2-hydroxyacids. Such analytical finding was considered correct and scored by 2 points. Moreover, 17 participants analysed urinary amino acids and 15 of them detected elevated excretion of branched-chain amino acids. The analytical performance for this sample was excellent (100%).

Interpretative proficiency: Maple syrup urine disease was considered correct diagnosis. 17 labs concluded the correct diagnosis so the interpretative proficiency for this sample was excellent (100%).

Recommendations: Confirmation of diagnosis by enzymatic assay and/or mutation analysis was considered helpful.

Overall impression: An easy sample with excellent total proficiency score (100%).

Sample E

Patient: The urinary sample was obtained from a 22-years old woman with hawkinsinuria, the sample was provided by Dr. Adolf Mühl from Neugeborenen Screening Labor of the Univ. Kinderklinik in Wien. Diagnosis in this patient was confirmed by molecular analyses.

Analytical performance: All participants analyzed urinary amino acids. Only seven reported elevated excretion of hawkinsin, such analytical finding was considered correct and scored by 1 point. Three participants reported unknown peak, such analytical finding was considered partially correct and scored by 0.5 point. All 17 participants performed analysis of organic acids. Thirteen participants observed the increased excretion of 4-hydroxy-cyclohexylacetate. Such analytical finding was considered correct and scored by 1 point. The analytical performance for this sample was below average (63%).

Interpretative proficiency: Diagnosis of hawkinsinuria was considered correct. The proficiency score of 65% was below the usual performance of our group.

Recommendations: Although further confirmation of hawkinsinuria is not necessary a confirmation of diagnosis by enzymatic assay and/or mutation analysis can be useful in case of prenatal diagnosis in the affected family.

Overall impression: Moderately difficult DPT sample with a slightly lower average proficiency score.

Sample F (common sample)

Patient: The sample was obtained from an 8-year old boy with mucopolysaccharidosis type IIIA (Sanflippo disease). The diagnosis was established by enzyme analysis. The sample was obtained from our repository.

Analytical performance: A report on elevated concentration of glycosaminoglycans together with an increased proportion of heparan sulphate was considered a correct analytical result. The increased concentration of GAG with missing evaluation of individual GAG fractions was scored as partially correct. Fourteen participants reported elevated excretion of urinary GAG but only ten participants evaluated also fractions by electrophoresis or TLC; all of them reported the presence of increased heparan sulphate fraction. In the appendix you can find an electrophoretogram produced in our laboratory with clearly increased fractions of heparan sulphate. The analytical performance was suboptimal reaching only 68 %.

Interpretative proficiency: The diagnosis of mucopolysaccharidosis type III (either alone or with other MPS types) was considered good 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 71%.

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 the 4 enzymes involved in heparan sulphate metabolism (heparan N-sulfatase, α-N-acetyl-glucosaminidase, acetyl-CoA:α-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulphatase) in leucocytes or cultured fibroblasts was considered helpful. For participants who only quantified GAG concentration the recommendation for electrophoresis or TLC was considered helpful.

Overall impression: Typical DPT sample with an average proficiency score.

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 30 points per year.

8. Score of participants for individual samples

Survey 2008/1

Lab no	Sample A Succinic semialdehyde dehydrogenase deficiency				Sample B No known IEM (epilepsy on treatment)				Sample C Adenylosuccinate lyase deficiency			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	2	1	1	**4**	0	2	1	**3**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
4	2	2	1	**5**	2	2	0	**4**	0	0	1	**1**
5	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
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**	2	2	1	**5**	0	0	0	**0**
9	0	0	0	**0**	2	2	0	**4**	0	0	0	**0**
10	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
11	2	2	1	**5**	2	2	0	**4**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	1	1	**4**	1	2	1	**4**
14	2	2	1	**5**	2	2	0	**4**	0	0	1	**1**
15	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
16	0	0	0	**0**	2	0	0	**2**	0	0	0	**0**
17	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
18	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**

Survey 2008/2

Lab no	Sample D Maple syrup urine disease				Sample E Hawkinsinuria				Sample F MPS type IIIA (Sanflippo disease)			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	1	2	1	**4**	0	0	0	**0**
2	2	2	1	**5**	1	0	0	**1**	0	0	0	**0**
3	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
4	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
5	2	2	1	**5**	1,5	2	1	**4,5**	0	0	1	**1**
6	2	2	1	**5**	0,5	0	0	**0,5**	2	2	1	**5**
7	2	2	1	**5**	0,5	0	0	**0,5**	2	2	1	**5**
8	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
9	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
10	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
11	2	2	1	**5**	1	2	1	**4**	1	1	1	**3**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
15	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
16	2	2	1	**5**	0	0	0	**0**	0	0	0	**0**
17	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**
18	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**

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

9. Total score of participants for individual surveys and their performance in 2008

Lab no	Survey 2008/1 [points]	Survey 2008/2 [points]	Total point 2008
1	12	9	**21**
2	15	6	**21**
3	10	15	**25**
4	10	14	**24**
5	10	10,5	**20,5**
6	15	10,5	**25,5**
7	15	10,5	**25,5**
8	10	15	**25**
9	4	10	**14**
10	14	15	**29**
11	14	12	**26**
12	15	15	**30**
13	13	15	**28**
14	10	14	**24**
15	10	13	**23**
16	2	5	**7**
17	10	11	**21**
18	0	0	**0**

10. Score summary in 2008

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
A	***Succinic semialdehyde dehydrogenase deficiency***	79	82	82	81
B	***No known IEM (epilepsy on treatment)***	100	88	71	86
C	***Adenylosuccinate lyase deficiency***	44	53	65	54
D	***Maple syrup urine disease***	100	100	100	100
E	***Hawkinsinuria***	63	65	65	64
F	***MPS type IIIA (Sanflippo disease)***	68	71	82	74

"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 more than 14 points within the calendar year are considered to be performing satisfactory, two participants did not meet this criterion and one participant did not return any results.

12. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Prague took place during the 44th Annual Symposium of SSIEM in Lisboa on 2nd September 2008, seven laboratories were represented. The following items were discussed during the annual meeting of our DPT centre:

1. Information
   - training course, meeting ERNDIM
   - ERNDIM is aiming at accrediting Schemes
   - Possible changes in DPT (sample recruitment and distribution, web based system at CSCQ)
   - 20% discount
2. Tests required for to 2008
   - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Submission of results
   - the participants approved the acceptance of 2008 results submitted past the deadline
4. Discussion of results of samples A-F
   - scoring of 2008 results proposed by organizer has been accepted

13. Changes planned in 2009

- ✓ Submission and evaluation of results and reporting via web: the system is now being developed by B. Fowler, P. Litynski and V. Kozich; testing of this system is now in a pilot phase. We thank all participants for their feedback in 2008 and we will ask you for cooperation in testing a more advanced version in 2009.

14. Tentative schedule of DPT scheme and fee in 2009

Sample distribution	March 9, Monday
Start of analysis of Survey 2009/1	March 23, Monday
Survey 2009/1 – results submission	April 10, Friday
Survey 2009/1 – report	May 8, Friday
Start of analysis of Survey 2009/2	June 8, Monday
Survey 2009/2 – results submission	June 26, Friday
Survey 2009/2 – report	August 28, Friday
Annual meeting of the participants	to be determined
Annual report 2009	November 30, Monday

Since there will be no SSIEM Symposium in 2009 (and ICIEM Symposium will take place in the USA) it is at present unclear when and where the next Annual Meeting of the DPT Center Prague will be organized.

The Executive Board of ERNDIM determined the DPT fee for 2009 in the amount of 290 €.

15. Certificate of participation in Proficiency Testing for 2008

The certificate of participation will be provided by the ERNDIM to all participants, who returned the results of both surveys.

Prague, February 5, 2009

Viktor Kožich, MD, PhD
Scientific Advisor to the Scheme
vkozich@lf1.cuni.cz

Petr Chrastina, M.Sc.
Scheme Organizer
petr.chrastina@lf1.cuni.cz