ERNDIM DPT Center Prague

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Proficiency Testing Centre Prague
Annual Report 2011

1. Introduction

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

2. Geographical distribution of participants

Nineteen laboratories from 13 countries have participated in our Diagnostic Proficiency Testing scheme in 2011, 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	2
Latvia	1
Malaysia	1
Poland	1
Slovakia	3
Switzerland	1
in total	**19**

3. Logistics of the scheme

- ✓ Two surveys: 2011/1 – samples A, B and C
  2011/2 – 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 DPT Amsterdam (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 2011: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2010

Sample distribution	April 26, Tuesday
Start of analysis of Survey 2011/1	May 9, Monday
Survey 2011/1 – results submission	May 27, Friday
Start of analysis of Survey 2011/2	June 6, Monday
Survey 2011/2 – results submission	June 24, Friday
Survey 2011/1 and 2 – report	August 12, Friday
Annual meeting of participants	August 30, Tuesday
Annual report 2011	November 28, Monday

5. The receipt of samples and results

Date of receipt of samples (samples sent on April 26, 2011)

date of receipt (reported by participants)	number of participants	date of receipt (reported by courier service)	number of participants
1 day	7	1 days	12
2 days	5	2 days	4
3 days	2	3 days	3
not indicated	5	-	-

Submission of results

	2011/1	2011/2
in time	17	18
3 days delay	1	-
no answer	1	1

6. Samples

Sample A

Patient: The sample was obtained from a 4 years old girl suffering from mucopolysaccharidosis type III A (morbus Sanfilippo). Enzymatic assay in leukocytes confirmed a total lack of heparan sulphamidase activity. The sample was taken from our repository.

Analytical performance: Elevated excretion of glycosaminoglycans and increased proportion of heparan sulphate were considered a correct analytical result. Increased excretion of GAGs without report on heparan sulphate elevation was scored as partially correct. Analytical performance was suboptimal (69) %.

Interpretative proficiency: The diagnosis of mucopolysaccharidosis type III 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 poor (69%).

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 (heparan-N sulfatase, N-acetylglucosaminidase, acetyl-CoA:alfa-glucosaminide-N-acetyltransferase, N-acetylglucosamine 6-sulfatase) in leukocytes 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 suboptimal proficiency score. The performance is much better that in year 2004, but it did not improve since 2007.

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
2004E	**Mucopolysaccharidosis type III A**	38	28	55	**37**
2007F	**Mucopolysaccharidosis type III A**	68	71	82	**74**
2011A	**Mucopolysaccharidosis type III A**	69	69	67	**70**

Sample B

Patient: This urinary sample was obtained from a patient without any known inborn error of metabolism suffering from seizures, 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 17 labs detected metabolites of valproic acid, seven 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. 17 labs reported increased excretion of glycine in urine, such analytical finding was also considered correct and scored by 1 point. The analytical performance for this sample was very good (94%).

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 scored with 0 points. The interpretative proficiency for this sample was good (89%).

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 good proficiency score.

Sample C

Patient: This sample came from a 9-years old male patient with D-2-hydroxyglutaric aciduria with macrocephaly, mental retardation and leukodystrophy. Absolute configuration of 2-hydroxyglutaric acid was established by ^{13}C high-resolution NMR spectra. Enzymatic activities of D-2-hydroxyglutarate dehydrogenase measurement as well as mutation analysis of the relevant gene are pending. This sample was contributed by Dr. Wanda Gradowska from the Children´s Memorial Health Institute in Warsaw.

Analytical performance: The presence of 2-hydroxyglutaric aciduria was considered correct analytical results. All labs have done analysis of organic acid and all reported elevated concentration of 2-hydroxyglutaric acid. The analytical performance of this sample was excellent (100%). Bacterial contamination (positive nitrites) of this sample did not affect the analytical findings.

Interpretative proficiency: 2-Hydroxyglutaric aciduria and/or specifically D-2-hydroxyglutaric aciduria were considered correct conclusions; such interpretation was scored 2 points. Diagnosis of L-2-hydroxyglutaric aciduria was considered partially misleading and was scored by 1 point. Fourteen participants suggested the correct diagnosis; four participants suggested L-hydroxyglutaric aciduria and one participant proposed glutaric aciduria type I. The final interpretative score was 89%.

Recommendations: A special enantiomeric analysis to distinguish L- and D-2-hydroxyglutaric acid is a crucial test. The recommendation of such analysis and/or enzymatic assay were considered helpful and scored by 1 point.
Overall impression: An easy sample from a patient with a rare IEM with an excellent total proficiency score (96%).

Sample D
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. The analytical performance was good (89%), only 2 labs failed to detect 4-hydroxybutyric acid.
Interpretative proficiency: Succinic semialdehyde dehydrogenase deficiency was considered correct diagnosis. The interpretative proficiency score was good (89%).
Recommendations: Confirmation of diagnosis by enzymatic assay and/or mutation analysis was considered helpful.
Overall impression: Typical DPT sample with a good proficiency score.

Sample E
Patient: This sample came from a 21-years old girl with argininosuccinic aciduria due to argininosuccinate lyase deficiency. The urine was collected during hospitalization; the patient is receiving specific treatment. The diagnosis was established by enzyme analysis. This sample was contributed by the Dr. Jeannette Klein from Charité-Campus Virchow – Klinikum in Berlin.
Analytical performance: The presence of argininosuccinic acid and its anhydrides was considered a correct result. It is pleasing that in contrast to previous circulations of argininosuccinic aciduria samples, this time only 1 lab was not able to identify argininosuccinate. The analytical performance of this sample was 94%.

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
2002E	**Argininosuccinic aciduria**	73	70	73	**72**
2007C	**Argininosuccinic aciduria**	76	79	82	**79**
2011D	**Argininosuccinic aciduria**	94	94	94	**94**

Interpretative proficiency: The diagnosis of argininosuccinic aciduria due to argininosuccinate lyase deficiency was considered good. The interpretative proficiency score for this sample was 94%.
Recommendations: Although further confirmation of argininosuccinic aciduria I 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: An easy sample with high total proficiency score (94%).

Sample F (common sample)
Patient: The common sample provided by the DPTC Rotterdam was obtained from a 7-year old girl with guanidinoacetate methyltransferase deficiency. The diagnosis is solely based on demonstrating the urinary excretion of guanidinoacetate. Enzyme and mutation analysis is pending.
Comment: This sample has to be considered as an educational sample, since the special guanidinoacetate analysis necessary for establishing the diagnosis is not required in our DPT scheme. Only five labs measured guanidinoacetate in this sample using a special assay. Four labs that detected elevated concentration of guanidinoacetate suggested the correct diagnosis.

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 2011/1

Lab no	Sample A Mucopolysaccharidosis type III A				Sample B No known IEM (epilepsy on treatment)				Sample C D-2-hydroxyglutaric aciduria			
	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	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	2	0	**4**	2	2	1	**5**
7	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
8	1	1	1	**3**	2	2	1	**5**	2	2	1	**5**
9	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
10	2	2	1	**5**	2	2	0	**4**	2	2	1	**5**
11	1	0	0	**1**	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	0	1	**3**	2	2	1	**5**
14	1	1	1	**3**	2	2	0	**4**	2	1	1	**4**
15	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
16	1	1	0	**2**	2	2	1	**5**	2	2	1	**5**
17	0	1	0	**1**	2	0	1	**3**	2	1	1	**4**
18	0	0	0	**0**	0	2	1	**3**	2	1	1	**4**
19	1	1	1	**3**	2	2	1	**5**	2	1	1	**4**

Survey 2011/2

Lab no	Sample D Succinic semialdehyde dehydrogenase deficiency				Sample E Argininosuccinic aciduria			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**
3	2	2	1	**5**	2	2	1	**5**
4	2	2	1	**5**	2	2	1	**5**
5	2	2	1	**5**	2	2	1	**5**
6	0	0	0	**0**	2	2	1	**5**
7	2	2	1	**5**	2	2	1	**5**
8	2	2	1	**5**	2	2	1	**5**
9	2	2	1	**5**	2	2	1	**5**
10	2	2	1	**5**	2	2	1	**5**
11	2	2	1	**5**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	2	2	1	**5**
15	0	0	0	**0**	0	0	0	**0**
16	2	2	1	**5**	2	2	1	**5**
17	2	2	1	**5**	2	2	1	**5**
18	2	2	1	**5**	2	2	1	**5**
19	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 2011

Lab no	Survey 2011/1 [points]	Survey 2011/2 [points]	Total point 2011
1	15	10	**25**
2	15	10	**25**
3	15	10	**25**
4	10	10	**20**
5	15	10	**25**
6	14	5	**19**
7	15	10	**25**
8	13	10	**23**
9	15	10	**25**
10	14	10	**24**
11	10	10	**20**
12	15	10	**25**
13	13	10	**23**
14	11	10	**21**
15	0	0	**0**
16	12	10	**22**
17	8	10	**18**
18	7	10	**17**
19	12	0	**12**

10. Score summary in 2011

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
A	***Mucopolysaccharidosis type III A***	69	69	67	70
B	***No known IEM (epilepsy on treatment)***	94	89	78	89
C	***D-2-hydroxyglutaric aciduria***	100	89	100	96
D	***Succinic semialdehyde dehydrogenase deficiency***	89	89	89	89
E	***Argininosuccinic aciduria***	94	94	94	94

"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

Since a sample F could not have been diagnosed using the methods requested in our scheme, the participants who obtained 15 or more points in the year 2011 are considered as satisfactory performers. Two participants did not reach the threshold of 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 2011 in Geneva on 30th August 2011, seven 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)
2. Tests required for to 2012
   - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Submission of results
   - the participants approved the acceptance of 2011 results submitted past the deadline
4. Discussion of results of samples A-E
   - scoring of 2011 results proposed by organizer has been accepted by participants

13. Tentative schedule of DPT scheme and fee in 2012

Sample distribution	March 26, Monday
Start of analysis of Survey 2012/1	April 16, Monday
Survey 2012/1 – results submission	May 4, 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	November 28, Monday

The annual meeting of participants will take place on September 4th during the Annual Symposium of SSIEM in Birmingham, UK.

The Executive Board and Board of Trustees of ERNDIM determined the DPT fee for 2012 in the amount of 334 €.

14. Certificate of participation and performance in Proficiency Testing for 2011

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

Prague, November 28, 2011

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