ERNDIM DPT Center Prague

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Proficiency Testing Centre Prague
Annual Report 2010

1. Introduction

In 2010 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 2010, for details see the below table:

Country	Number of participants
Austria	1
Croatia	1
Cyprus	1
Czech Republic	1
Denmark	1
Finland	1
France	1
Germany	4
Greece	2
Latvia	1
Malaysia	1
Poland	1
Slovakia	2
Switzerland	1
in total	**19**

3. Logistics of the scheme

- ✓ Two surveys: 2010/1 – samples A, B and C
  2010/2 – samples D, E and F

  Origin of samples: Five urines obtained from patients with known diagnoses (samples were provided by organizers) + a common sample from DPT Lyon (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 2010: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2010

Sample distribution	March 29, Monday
Start of analysis of Survey 2010/1	April 12, Monday
Survey 2010/1 – results submission	April 30, Friday
Survey 2010/1 – report	May 28, Friday
Start of analysis of Survey 2010/2	June 7, Monday
Survey 2010/2 – results submission	June 25, Friday
Survey 2010/2 – report	August 13, Friday
Annual meeting of participants	August 31, Tuesday
Annual report 2010	November 29, Monday

5. The receipt of samples and results

Date of receipt of samples (samples sent on March 29, 2010)

date of receipt (reported by participants)	number of participants	date of receipt (reported by courier service)	number of participants
1 day	6	1 days	11
2 days	2	2 days	4
3 days	2	3 days	4
4 days	1	-	-
not indicated	8	-	-

Submission of results

	2010/1	2010/2
in time	17	17
1 day delay	1	-
3 days delay	1	-
4 days delay	-	1
7 days delay	-	1
no answer	-	-

6. Samples

Sample A

Patient: A 11 year old boy with aminoacylase 1 deficiency. The diagnosis was established by demonstrating enzyme deficiency in lymphocytes and completed by molecular analysis. This sample was contributed by the Dr. Wanda Gradowska from the Laboratory Diagnostics Department in Warsaw.

Analytical performance: Increased excretion of N-acetylated amino acids (N-acetylalanine, N-acetylmethionine, N-acetylglycine, N-acetylglutamine, N-acetylglutamate, N-acetylserine, N-acetylvaline, N-acetylleucine, N-acetylisoleucine, N-acetylthreonine) with normal excretion of N-

acetylaspartate was considered a correct analytical result. The analytical performance was rather poor reaching only 58%.
Interpretative proficiency: Aminoacylase 1 deficiency was considered a correct diagnosis. The interpretative proficiency score for this sample in laboratories that detected increased excretion of N-acetylated amino acids was good. The interpretative performance of 58% was below the usual performance of our group
Recommendations: Confirmation of diagnosis by enzyme assay of aminoacylase 1 activity in fibroblasts or leucocytes and/or mutation analysis of *ACY1* gene was considered helpful.
Overall impression: Difficult sample with total proficiency score of 58%, which was lower than for the other samples in this survey.

Sample B
Patient: A 78 year old woman with xanthinuria due to xanthine oxidase deficiency. The diagnosis was confirmed by molecular genetic analysis. This sample was contributed by the Dr. Wanda Gradowska from the Laboratory Diagnostics Department in Warsaw.
Analytical performance: Fifteen laboratories reported the results of purines/pyrimidines analysis, some of them performed this analysis in a cluster with another lab. Increased levels of xanthine were considered a correct analytical result. The analytical performance of this sample was 74%.
Interpretative proficiency: Diagnosis of xanthinuria due to xanthine dehydrogenase deficiency was considered correct. One lab suggested a disorder of purine/pyrimidine metabolism, however, no relevant laboratory test was carried out - this partially correct conclusion was therefore scored by 0 points. The interpretative performance for this sample in laboratories that detected xanthine was good, overall performance was slightly suboptimal (74%).
Recommendations: Confirmation of diagnosis by enzyme assay of xanthine oxidase in liver or jejunal or duodenal mucosa biopsy sample (although these tests would not be carried out in practice) and/or mutation analysis of *XHD* gene was considered helpful. Recommendation to carry out analysis of purines and pyrimidines for those participants that did not perform P/P analysis was considered also helpful.
Overall impression: Moderately difficult DPT sample with an average proficiency score.

Sample C
Patient: A 7 year old girl with methylmalonic acidemia due to the deficiency of methylmalonyl-CoA mutase. The diagnosis was confirmed by demonstrating enzyme deficiency in lymphocytes and completed by molecular analysis. The sample was obtained from our repository.
Analytical performance: All participants analyzed organic acids and demonstrated increased excretion of methylmalonate which was considered a good analytical performance.
Interpretative proficiency: The diagnosis of methylmalonic acidemia was considered correct. The proficiency score for this sample was excellent (100%).
Recommendations: Confirmation of diagnosis by enzyme assay of methylmalonyl-CoA mutase activity in fibroblasts or leucocytes and/or mutation analysis of the *MUT* gene was considered helpful.
Overall impression: An easy sample with an excellent total proficiency score (100%).

Sample D
Patient: A 6 year old girl with multiple acyl-CoA dehydrogenase deficiency. The diagnosis was established by demonstrating ETF dehydrogenase deficiency in fibroblasts. The sample was obtained from our repository.
Analytical performance: All participants analyzed organic acids. Eighteen labs demonstrated increased excretion of ethylmalonic acid and/or glutaric acid and/or 2-hydroxyglutaric acids and/or glycine conjugates, which were all considered a correct analytical result. One lab demonstrated increased excretion of glutaric acid and 2-hydroxyglutaric acids, which was considered partially correct.

Interpretative proficiency: The diagnosis of multiple acyl-CoA dehydrogenase deficiency was considered correct. Participant who proposed two completely different diagnosis (e.g. OTC deficiency or GAII) was scored with only one point. The proficiency score for this sample was good (84%).
Recommendations: Confirmation of diagnosis by ETF-QO and ETF activity in muscle or fibroblasts and/or mutation analysis of ETFDH, ETFA and ETFB genes was considered helpful.
Overall impression: An easy sample with a good total proficiency score (87%).

Sample E
Patient: A 7 months old girl with GM1-gangliosidosis type I due to β-galactosidase deficiency. The diagnosis was established by demonstrating enzyme deficiency in lymphocytes and completed by molecular analysis. This sample was contributed by the Dr. Darina Behulova from Department of Clinical Biochemistry of University Children's Hospital in Bratislava.
Analytical performance: The pattern of oligosaccharides (OLS) characteristic for GM1-gangliosidosis was considered a correct analytical finding. An abnormal OLS pattern was considered partially correct. The analytical performance was slightly suboptimal (74%).
Interpretative proficiency: The diagnosis of GM1-gangliosidosis was considered correct. One lab suggested a lysosomal storage disease based purely on clinical grounds, and one lab suggested GM1-gangliosidosis, however, no relevant laboratory test was carried out - these conclusions were therefore scored by 1 points. The interpretative proficiency score for this sample was slightly suboptimal (79%).
Recommendations: Confirmation of diagnosis by enzyme assay of β-galactosidase activity in leucocytes or fibroblasts and/or mutation analysis of the *GLB1* gene were considered helpful.
Overall impression: The analytical and interpretative performance for this sample with characteristic oligosaccharide profile demonstrates persistent difficulties in diagnosing abnormal OLS patterns. Rather easy DPT sample with slightly suboptimal (80%) proficiency score.

Sample F (common sample)
Patient: The common sample provided by the DPTC Lyon was obtained from a 43 year old woman with a lysosomal storage disease – sialidosis due to α-neuraminidase deficiency. Diagnosis was confirmed by measurement of α-neuraminidase activity in leukocytes and fibroblasts while β-galactosidase activity was normal.
Analytical performance: The pattern of OLS and/or sialylOLS characteristic for sialidosis was considered a correct analytical finding. Abnormal OLS pattern without specified diagnosis was considered partially correct. The analytical performance was slightly suboptimal (71%). Two participants reported elevated excretion of bound sialic acid, which was also considered a correct result.
Interpretative proficiency: The diagnosis of sialidosis (α-neuraminidase deficiency) was considered correct. Thirteen laboratories reached correct diagnosis and one lab reported possible storage disease (partially correct). Two labs suggested diagnosis of sialidosis based purely on clinical grounds with no relevant laboratory test being performed and one lab suggested diagnosis of sialidosis based on incorrect laboratory test (increased excretion of free sialic acid) - this conclusion were therefore scored by 1 points. The interpretative proficiency score for this sample was suboptimal (79%).
Recommendations: Confirmation of diagnosis by enzyme assay of α-neuraminidase activity preferably in fibroblasts and/or mutation analysis of *NEU1* gene were considered helpful.
Overall impression: The analytical and interpretative performance for this sample suggests possible difficulties in diagnosing abnormal OLS pattern. Moderately difficult DPT sample with slightly suboptimal (80%) proficiency score.

7. Scoring of results

There is a new procedure for scoring DPT Scheme. Scores has been reviewed by two independent advisors from two DPT Centres and final scoring is provided after discrepancies have been resolved. Results of the reviewing process and the procedure for solving discrepancies were discussed at the ERNDIM Scientific Advisory Board meeting in Lyon in April 2011.
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 20010/1

Lab no	Sample A Aminoacylase 1 deficiency				Sample B Xanthinuria				Sample C Methylmalonic acidemia			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	0	0	0	**0**	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**	0	0	0	**0**	2	2	1	**5**
7	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
8	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
9	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
10	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
11	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
12	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
14	0	0	0	**0**	0	0	1	**1**	2	2	1	**5**
15	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
16	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
17	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
18	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
19	0	0	0	**0**	0	0	0	**0**	2	2	1	**5**

Survey 2010/2

Lab no	Sample D Multiple acyl-CoA dehydrogenase deficiency				Sample E GM1-gangliosidosis type I				Sample F Sialidosis type I			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
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	2	2	1	**5**	0	1	1	**2**	0	1	1	**2**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
7	2	2	1	**5**	0	0	1	**1**	0	0	1	**1**
8	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
9	1	1	1	**3**	2	2	1	**5**	2	2	1	**5**
10	2	2	1	**5**	1	0	1	**2**	0	1	1	**2**
11	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
12	2	1	0	**3**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
14	2	2	0	**4**	2	2	1	**5**	2	2	1	**5**
15	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
16	2	1	1	**4**	1	2	1	**4**	1	2	1	**4**
17	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
18	2	2	1	**5**	0	1	1	**2**	0	1	1	**2**
19	2	2	1	**5**	1	0	0	**1**	1	0	1	**2**

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

9. Total score of participants for individual surveys and their performance in 2010

Lab no	Survey 2010/1 [points]	Survey 2010/2 [points]	Total point 2010
1	10	13	**23**
2	15	15	**30**
3	10	15	**25**
4	10	9	**19**
5	15	15	**30**
6	10	15	**25**
7	11	7	**18**
8	15	15	**30**
9	10	13	**23**
10	15	9	**24**
11	15	14	**29**
12	10	13	**23**
13	15	15	**30**
14	6	14	**20**
15	15	15	**30**
16	11	12	**23**
17	10	15	**25**
18	15	9	**24**
19	5	8	**13**

10. Score summary in 2010

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
A	***Aminoacylase 1 deficiency***	58	58	58	58
B	***Xanthinuria***	74	74	89	77
C	***Methylmalonic acidemia***	100	100	100	100
D	***Multiple acyl-CoA dehydrogenase deficiency***	89	84	89	87
E	***GM1-gangliosidosis type I***	74	79	95	79
F	***Sialidosis type I***	71	79	100	80

"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 in the year 2010 are considered as satisfactory performers, one participant 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 2010 in Istanbul on 31st August 2010, eight 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
   - changes in DPT (sample recruitment and distribution, web based system at CSCQ)
2. Tests required for to 2011
   - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Submission of results
   - the participants approved the acceptance of 2010 results submitted past the deadline
4. Discussion of results of samples A-F
   - scoring of 2010 results proposed by organizer has been accepted

13. Tentative schedule of DPT scheme and fee in 2011

Sample distribution	April 26, Tuesday
Start of analysis of Survey 2011/1	May 9, Monday
Survey 2011/1 – results submission via web	May 27, Friday
Start of analysis of Survey 2011/2	June 6, Monday
Survey 2011/2 – results submission via web	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

The annual meeting of participants will take place on August 30th during the Annual Symposium of SSIEM in Geneva, Switzerland.

The Executive Board and Board of Trustees of ERNDIM determined the DPT fee for 2011 in the amount of 326 €.

14. Certificate of participation and performance in Proficiency Testing for 2010

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

Prague, May 3, 2011

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