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: ++420/224 967 647, 224 967 679
fax: ++420/224 967 081 or 224 967 119

Proficiency Testing Centre Prague
Annual Report 2007

1. Introduction

In 2007 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 2007, 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: 2007/1 – samples A, B and C
  2007/2 – samples D, E and F

Origin of samples: Five urines were obtained from patients with known diagnoses (samples were provided by the DPTC participants and by the organizers) and a common ERNDIM DPT sample was obtained from Sheffield. The samples with the exception of the common sample F 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. Jeannette Klein for providing samples for 2007 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 2007: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2007

Sample distribution	March 19, Monday
Start of analysis of Survey 2007/1	March 26, Monday
Survey 2007/1 – results submission	April 17, Tuesday
Survey 2007/1 – report	May 31, Thursday
Start of analysis of Survey 2007/2	May 28, Monday
Survey 2007/2 – results submission	June 18, Monday
Survey 2007/2 – report	July 19, Tuesday
Annual meeting of participants	September 4, Tuesday
Annual report 2007	February 27, 2008

5. The receipt of samples and results

Date of receipt of samples (samples sent on March 19, 2007)

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

Deadlines of the results submission

	2007/1	2007/2
in time	14	14
1 day delay	1	1
2 days delay	-	1
3 days delay	-	1
8 days delay (reason not given)	1	-
10 days delay (equipment malfunction)	1	-
no answer	1	1

6. Samples

Sample A

Patient: The sample was obtained from 13-years old boy with mucopolysaccharidosis type VI (morbus Maroteaux-Lamy). The diagnosis was established by enzyme analysis and completed by molecular analysis. This sample was contributed by the Dr. Jeannette Klein from Charité- Campus Virchow – Klinikum in Berlin.

Analytical performance: A report on elevated concentration of glycosaminoglycans together with an increased proportion of dermatan sulphate was considered a correct analytical result. The

increased concentration of GAG with missing evaluation of individual GAG fractions was scored as partially correct; report of "abnormal pattern" or "pathological pattern" without further specification of unusual fractions was considered unsatisfactory. Sixteen participants reported elevated excretion of urinary GAG but only fourteen participants evaluated also fractions by electrophoresis or TLC; thirteen of them reported the presence of increased dermatan sulphate fraction. In the appendix you can find an electrophoretogram produced in our laboratory with clearly increased fractions of dermatan sulphate. The analytical performance was 85 %.

Interpretative proficiency: The diagnosis of mucopolysaccharidosis type VI (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. At the next Annual Meeting we should discuss whether the latter types of conclusions should be considered partially helpful or wrong answers. The interpretative proficiency score for this sample was good (85%).

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 (N-acetylgalactosamine-4-sulphatase , α-L-iduronidase, iduronate-2-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. For one participant who did not perform any GAG analysis the recommendation to do GAG analysis was considered helpful.

Overall impression: Typical DPT sample with average proficiency score.

Sample B

Patient: The sample was obtained from 9-years old girl with cystinuria. The diagnosis was originally based on urinary examination of amino acids.

Analytical performance: Seventeen participants analyzed amino acids and all of them detected increased cystine, ornithine, lysine and arginine in qualitative or quantitative amino acids analysis. Such finding was considered a good analytical performance. The analytical performance for this sample was excellent (100%).

Interpretative proficiency: The diagnosis of cystinuria was considered correct. 17 labs concluded the correct diagnosis so the interpretative proficiency for this sample was excellent (100%).

Recommendations: Although further confirmation of cystinuria is not necessary DNA studies might help clarifying the exact type of cystinuria and aid in the identification of carriers. We accepted all recommendations.

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

Sample C

Patient: This sample came from 21-years old girl with argininosuccinic aciduria due to argininosuccinate lyase deficiency. The urine was collected during hospitalization; the patient is under 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 disappointing that only 13 labs were able to identify argininosuccinate. The analytical performance of this sample was only 76%.

Interpretative proficiency: The diagnosis of argininosuccinic aciduria due to argininosuccinate lyase deficiency was considered good while suspicion for urea cycle disorder was considered helpful but incomplete. The interpretative proficiency score for this sample was 79%.

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 suboptimal total proficiency score (79 %).

Sample D

Patient: The sample was obtained from a 10-years old boy with mucopolysaccharidosis type IVA (morbus Morquio A) and adenine phosphoribosyltransferase deficiency (APRT deficiency). The diagnosis was established by enzyme analysis and completed by molecular analysis.

Analytical performance: The participants were expected to detect analytes relating to both MPS type IVA and APRT deficiency. A report on elevated concentration of glycosaminoglycans together with an increased proportion of keratan sulphate was considered a correct analytical result for diagnosis of mucopolysaccharidosis type IVA (1 point). The increased concentration of GAG with missing evaluation of individual GAG fractions was scored as partially correct (0.5 point). Thirteen participants reported elevated excretion of urinary GAG but only nine participants evaluated also fractions by electrophoresis or TLC; five of them reported the presence of increased keratan sulphate fraction. In the appendix you can find an electrophoretogram produced in our laboratory with clearly increased fractions of keratan sulphate. Only 11 laboratories reported the results of purines/pyrimidines analysis, some of them performed this analysis in cluster with another lab. Increased levels of 2,8-dihydroxyadenine and/or adenine were considered a correct analytical result for diagnosis of APRT deficiency (1 point). The analytical performance was 51 %, which is congruent with the presence of two IEMs.

Interpretative proficiency: The diagnosis of mucopolysaccharidosis type IV or IVA and APRT deficiency was considered good while suspicion for MPS, MPS IV only or for APRT deficiency only were considered helpful but incomplete. The interpretative proficiency score for this difficult sample was 47%.

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 (N-acetylgalactosamine-6-sulphatase, β-galactosidase) in leucocytes or cultured fibroblasts and mutation analysis of GALNS gene was considered helpful. For participants who only quantified GAG concentration the recommendation for electrophoresis or TLC was considered helpful. Enzyme assays of adenine phosphoribosyltransferase in erythrocytes and/or mutation analysis of APRT gene for diagnosis APRT deficiency was considered helpful.

Overall impression: A difficult sample from a patient with two IEMs. The total proficiency score of 50% was less satisfactory than for the other samples in this survey.

Sample E

Patient: The sample was obtained from an 8-years old girl with severe ornithine transcarbamylase (OTC) deficiency. The diagnosis was established by molecular analysis.

Analytical performance: The presence of increased concentrations of orotic acid and uracil was considered a correct analytical result regardless of the employed technique. The analytical performance for this sample was good (85%).

Interpretative proficiency: The diagnosis of OTC deficiency was considered good while suspicion for urea cycle disorder was considered helpful but incomplete. The interpretative proficiency score for this sample was 76%.

Recommendations: In our opinion the most important advice for follow-up investigation is the mutation analysis of the *OTC* gene, which was scored as helpful. Allopurinol loading test and amino acid profile in plasma for differentiation of citrulinemia were also scored as helpful. Enzyme assay of ornithine transcarbamylase in the liver does not seem to be appropriate for confirming diagnosis in heterozygouse carriers due to X-inactivation and also due to ethical consideration.

Overall impression: An easy sample with average total proficiency score (81%).

Sample F (common sample)

Patient: This sample came from 18-years old girl with α-aminoacid semialdehyde synthase deficiency. The diagnosis was based on urinary examination of amino acids.

Analytical performance: Seventeen participants analyzed amino acids. All of them detected increased lysine and only seven demonstrated presence of saccharopine in urine.
Finding of saccharopine and increased level of lysine in urine was considered a good analytical performance. The presence of increased lysine in AA analysis was considered only partially correct. The analytical performance of this sample was 74%.
Interpretative proficiency: The diagnosis of α-aminoacid semialdehyde synthase deficiency, hyperlysinemia I or II, saccharopinuria, hyperlysinemia or lysinuria was considered good. The interpretative proficiency score for this sample was good (88%).
Recommendations: Although further confirmation of α-aminoacid semialdehyde synthase deficiency is not necessary a confirmation of diagnosis by enzymatic assay and/or mutation analysis can be useful. Also amino acid profile in plasma was scored as helpful.
Overall impression: A typical sample with average total proficiency score (85 %).

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 2007/1

Lab no	Sample A MPS type VI				Sample B Cystinuria				Sample C Argininosuccinic aciduria			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	0	1	1	**2**	2	2	1	**5**	2	2	1	**5**
2	1	1	1	**3**	2	2	1	**5**	0	0	0	**0**
3	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
4	2	2	1	**5**	2	2	1	**5**	0	1	1	**2**
5	2	2	0	**4**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
7	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
8	2	2	1	**5**	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	1	**5**	2	2	1	**5**
11	0	1	1	**2**	2	2	1	**5**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	1	0	**3**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
15	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
16	2	1	0	**3**	2	2	1	**5**	0	0	0	**0**
17	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
18	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**

Survey 2007/2

Lab no	Sample D MPS type IVA and APRT deficiency				Sample E OTC deficiency				Sample F α-aminoacid semialdehyde synthase deficiency			
	A	**I**	**R**	**T**	**A**	**I**	**R**	**T**	**A**	**I**	**R**	**T**
1	0	1	0	**1**	1	2	1	**4**	1	2	1	**4**
2	2	2	1	**5**	2	1	1	**4**	1	0	1	**2**
3	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
4	1	1	0,5	**2,5**	2	2	1	**5**	2	2	1	**5**
5	1,5	1	1	**3,5**	2	0	1	**3**	1	2	1	**4**
6	1	2	1	**4**	2	2	1	**5**	1	2	1	**4**
7	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
8	0,5	0	0	**0,5**	1	2	1	**4**	2	2	1	**5**
9	0,5	0	0	**0,5**	1	1	1	**3**	2	2	1	**5**
10	1,5	1	0,5	**3**	2	2	1	**5**	2	2	1	**5**
11	1,5	1	1	**3,5**	2	2	1	**5**	1	0	0	**1**
12	1,5	1	1	**3,5**	2	2	1	**5**	2	2	1	**5**
13	0	0	0	**0**	2	2	0	**4**	2	2	1	**5**
14	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
15	1,5	1	1	**3,5**	2	0	0	**2**	1	2	1	**4**
16	0,5	1	0	**1,5**	0	0	0	**0**	2	2	1	**5**
17	0,5	0	0	**0,5**	2	2	1	**5**	1	2	1	**4**
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 2007

Lab no	Survey 2007/1 [points]	Survey 2007/2 [points]	Total point 2007
1	12	9	**21**
2	8	11	**19**
3	15	14	**29**
4	12	12,5	**24,5**
5	14	10,5	**24,5**
6	10	13	**23**
7	15	14	**29**
8	15	9,5	**24,5**
9	15	8,5	**23,5**
10	15	13	**28**
11	12	9,5	**21,5**
12	15	13,5	**28,5**
13	13	9	**22**
14	15	10	**25**
15	15	9,5	**24,5**
16	8	6,5	**14,5**
17	15	9,5	**24,5**
18	0	0	**0**

10. Score summary in 2007

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
A	***MPS type VI***	85	85	82	84
B	***Cystinuria***	100	100	100	100
C	***Argininosuccinic aciduria***	76	79	82	79
D	***MPS type IVA and APRT deficiency***	51	47	53	50
E	***OTC deficiency***	85	76	82	81
F	***α-aminoacid semialdehyde synthase deficiency***	74	88	94	85

"Easy" and "difficult" samples were included in the surveys. The analytical and interpretative performance was good to very good for most diagnoses while one sample was extremely difficult (diagnosis of both MPS type IVA and APRT deficiency in one patient).

11. Satisfactory performance

The participants who obtained more than 14 points within the calendar year are considered to be performing satisfactory. One participant did not return any results.

12. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Eastern Europe took place during the 43rd Annual Symposium of SSIEM in Hamburg on 4th September 2007, eight laboratories were represented. The following items were discussed during the annual meeting of our DPT centre:

1. Information
    - Eurogetest promotes accreditation of QA schemes
    - ERNDIM is aiming at accrediting Schemes
    - Possible changes in DPT (sample recruitment and distribution, web based system at CSCQ)
    - Increase in number of Schemes-pilots
2. Tests required for to 2008
    - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Shipment of samples
    - using the courier FedEx in the next year except shipments to Czech Republic and Slovakia
4. Submission of results
    - the participants approved the acceptance of 2007 results submitted past the deadline
5. Samples
    - it was emphasised by participants that more urine is needed for analyses
6. Discussion of results of samples A-F
    - scoring of 2007 results proposed by organizer has been accepted
    - Mucopolysaccharidoses: the participants concluded that incorrect types of mucopolysaccharidosis in Conclusions should be considered partially helpful and scored by one point

13. Changes planned in 2008

- ✓ 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 will be possible by help of participants from our centre – participants will be notified in due course.

14. Tentative schedule of DPT scheme and fee 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	January 16, 2009, Friday

Next annual meeting of participants will take place on September 2nd during the 44th Annual Symposium of SSIEM in Lisboa, Portugal.

The Executive Board of ERNDIM determined the fee for 2008 in the amount of 284 €.

15. Certificate of participation in Proficiency Testing for 2007

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

Prague, February 27, 2008

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

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