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: ++420/224 947 161, 224 967 679
fax: ++420/224 967 081 or 224 967 119

Proficiency Testing Centre Czech Republic
Annual Report 2015

1. Introduction

In 2015 proficiency testing in our centre was running as a regular ERNDIM scheme.

2. Geographical distribution of participants

Twenty laboratories from 14 countries have participated in the Diagnostic Proficiency Testing scheme in 2015, 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	5
Latvia	1
Malaysia	1
Philippines	1
Poland	1
Portugal	1
Slovakia	3
in total	**20**

3. Logistics of the scheme

- ✓ Two surveys: 2015/1 – samples A, B and C
  2015/2 – 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 the DPTC France (distributed in all five DPT schemes).
- ✓ In 2015 the samples with addition of thiomersal have been heat-treated and with the exception of the common sample D were re-analyzed in our Institute after receiving the samples from CSCQ that were shipped via courier at ambient temperature (to mimic

possible changes that might arise during transport). In all five samples prepared and checked by us the typical metabolic profiles were preserved after undergoing this treatment.

- ✓ The samples for Diagnostic Proficiency Testing scheme were distributed via CSCQ in Geneva. On 31st March 2015 the urinary samples were distributed to the participants at ambient temperature using the courier. Based on the report of the courier all parcels were delivered within 3 days.
- ✓ 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 2015: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2015

Sample distribution	March 31, Monday
Start of analysis of Survey 2015/1	April 7, Monday
Survey 2015/1 – results submission	April 30, Thursday
Survey 2015/1 – report	May 29, Friday
Start of analysis of Survey 2015/2	June 1, Monday
Survey 2015/2 – results submission	June 22, Monday
Survey 2015/2 – report	July 28, Tuesday
Annual meeting of participants	September 1, Tuesday
Annual report 2015	March 2016

5. Submission of results

	2015/1	**2015/2**
in time	20	20

6. Samples

Sample A

This sample was obtained from a 3 days old boy with argininosuccinic aciduria due to argininosuccinate lyase deficiency. The urine was collected during hospitalization; the patient was receiving specific treatment. The diagnosis is solely based on demonstrating the urinary excretion of argininosuccinic acid and its anhydrides. This sample was contributed by the Dr. Darina Behulova from Department of Clinical Biochemistry of University Children's Hospital in Bratislava.

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, analytical performance is improving. This time only 1 lab was not able to identify argininosuccinate. The analytical performance of this sample was 95% compared to 73% in the year 2002, for details see table below.

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**
2015A	**Argininosuccinic aciduria**	95	95		**95**

Interpretative proficiency and recommendation: The diagnosis of argininosuccinic aciduria due to argininosuccinate lyase deficiency was considered appropriate. Although further confirmation of argininosuccinic aciduria is not necessary a confirmation of diagnosis by enzymatic assay and/or mutation analysis may be useful in case of prenatal diagnosis in the affected family. The interpretative proficiency score for this sample was 95%.
Critical errors: The failure to recognize abnormal excretion of argininosuccinic acid and its anhydrides is considered by the ERNDIM SAB as a critical error which would prevent establishing the correct diagnosis; critical error was assigned to one participant in our scheme.
Overall impression: Easy DPT sample with very good proficiency score.

Sample B
Patient: This sample came from a 20 years old man with sialidosis type I due to neuraminidase deficiency. The diagnosis was established by demonstrating enzyme deficiency in cultured fibroblast. This sample was contributed by Dr. Ksenija Fumić from the Clinical Institute of Laboratory Diagnosis in Zagreb.
Analytical performance: The pattern of OLS and/or sialylOLS characteristic for sialidosis was considered a correct analytical finding. Abnormal OLS pattern characteristic for other glycoproteinoses or abnormal OLS pattern without specified diagnosis were considered partially correct. The analytical performance was slightly suboptimal (78%).
Interpretative proficiency and recommendation: The diagnosis of sialidosis due to alfa-neuraminidase deficiency was considered correct. Fourteen laboratories reached correct diagnosis. Confirmation of diagnosis by measurement of alfa-neuraminidase in leukocytes or cultured fibroblasts and/or mutation analysis was considered helpful. The diagnosis of GM1 gangliosidosis or other lysosomal storage disorders was considered partial correct. The interpretative proficiency score for this sample was good (83%).
Critical errors: The ERNDIM SAB did not assign any critical error for this sample.
Overall impression: Moderately difficult DPT sample with good proficiency score. All labs performing OLS analysis established correct diagnosis or at least an abnormal pattern; however, two labs did not analyse OLS.

Sample C
Patient: The sample was obtained from an 8 years old boy with cystinuria. The diagnosis was established by molecular analysis. The sample was obtained from our repository.
Analytical performance: The presence of cystinuria and dibasic hyperaminoaciduria were considered a correct analytical result and scored by 1 point for each. The analytical performance was very good (95%).
Interpretative proficiency and recommendation: Cystinuria or cystinuria/lysinuric protein intolerance were considered the correct diagnosis. The diagnosis of lysinuric protein intolerance alone was scored with 1 point. Confirmation of diagnosis by mutation analysis was considered helpful. The proficiency score for this sample was good (83%).
Critical errors: The failure to detect cystine and/or dibasic amino acids is considered by the ERNDIM SAB as a critical error which would prevent establishing the correct diagnosis; no critical error was observed in our scheme.
Overall impression: Easy DPT sample with good proficiency score.

Sample D (common sample)

The common sample provided by the DPTC France was obtained from a 34 years old woman with cystathionine beta-synthase deficiency. Further details on this sample are not available at the time of writing the report.

Analytical performance: All participants performed analysis of amino acids. 19 participants observed increased excretion of homocystine, such analytical finding was considered correct and scored by 2 points. Six participants also detected elevated excretion of methionine while seven reported normal excretion and one participant interpreted methionine excretion as decreased. This data indicate possible problems with reference ranges of methionine in urine. The analytical performance for homocystine was very good (95%).

Interpretative proficiency and recommendation: The diagnosis of homocystinuria due to CBS deficiency was considered correct while suspicion for remethylation types of homocystinuria was considered helpful but incomplete, mostly due to overinterpretation of analytical findings. The most important advice for follow-up investigation included the following recommendations: a/ total homocysteine and amino acids (methionine) in plasma and b/ CBS activity measurement and/or mutation analysis. The interpretative proficiency score for this sample was good (80%).

Critical errors: The failure to recognize abnormal homocystine excretion is considered by ERNDIM SAB as a critical error which would prevent establishing the correct diagnosis; critical error was assigned to one participant in our scheme.

Overall impression: Typical DPT sample with good total proficiency score (88%) although some participants overinterpreted methionine excretion and suggested remethylation defect (including the common MTHFR c.677C>T variant which is unlikely to lead to such degree of urinary homocysteine excretion).

Sample E

Patient: This sample was obtained from a 19 years old man suffering from mucopolysaccharidosis type IIIC due to deficiency of acetyl-CoA:alpha-glucosaminide N-acetyltransferase. The diagnosis was confirmed by enzymatic analysis. The sample was taken from our repository.

Analytical performance: Elevated excretion of glycosaminoglycans and increased proportion of heparan sulfate were considered a correct analytical result. Increased excretion of GAGs without reporting heparan sulfate elevation was scored as partially correct. The analytical performance of this sample was 90% compared to 38% in the year 2004.

Sample	Diagnosis	Analytical [%]	Interpretative [%]	Recommen-dations [%]	Total [%]
2004E	**MPS type III A**	38	28	55	**37**
2007F	**MPS type III A**	68	71	82	**74**
2011A	**MPS type III A**	69	69	67	**70**
2015E	**MPS type III C**	90	83		**86**

Interpretative proficiency and recommendation: 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. Confirmation of diagnosis by measurement of acetyl-CoA:alpha-glucosaminide N-acetyltransferase in leukocytes or cultured fibroblasts and/or mutation analysis was considered helpful. The interpretative proficiency score for this sample was good (83%).

Critical errors: The failure to detect elevated excretion of glycosaminoglycans is considered by the ERNDIM SAB as a critical error which would prevent establishing the correct diagnosis; no critical error was observed in our scheme.

Overall impression: Typical DPT sample with good total proficiency score (86%).

Sample F

Patient: This sample was obtained from a 4 years old boy with propionic acidemia. The diagnosis is solely based on demonstrating the urinary excretion of specific metabolites. This sample was contributed by Dr. Wanda Gradowska from the Children´s Memorial Health Institute in Warsaw.

Analytical performance: All participants performed analysis of organic acids. All participants observed the increased excretion of methylcitrate, such analytical finding was considered correct and scored by 1 point. 19 participants detected also elevated excretion of 3-hydroxypropionate, such analytical finding was also considered correct and scored by 1 point. The analytical performance was very good (98%).

Interpretative proficiency and recommendation: Propionic acidemia was considered the correct diagnosis. Confirmation of diagnosis by enzyme assay of propionyl-CoA carboxylase activity in fibroblasts or lymphocytes and/or mutation analysis was considered helpful. The proficiency score for this sample was very good (95%).

Critical errors: The failure to detect elevated excretion of methylcitrate and/or 3-hydroxypropionate is considered by the ERNDIM SAB as a critical error which would prevent establishing the correct diagnosis; no critical error was observed in our scheme.

Overall impression: Easy DPT sample with very good total proficiency score (96%).

7. Scoring of results

Two criteria are evaluated: analytical and interpretative proficiency. The recommendations pertaining to further investigations are scored as a part of interpretative proficiency. The summary of scoring criteria is given below.

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory or misleading (in some instances will be evaluated also as a critical error)	0
I	Interpretative proficiency	Good (diagnosis was established and appropriate further tests were recommended)	2
		Helpful but incomplete	1
		Misleading/wrong diagnosis (will be most likely evaluated also as a critical error)	0

The total score is calculated as a sum of these two criteria. The maximum that can be achieved is 4 points per sample, i.e. 12 points per survey and 24 points in 2015. Scores assigned by organizer and agreed at the Annual Meeting have been reviewed by an independent advisor from another DPT Centre and scoring was finalized after any possible discrepancies had been resolved at the March 2016 ERNDIM Scientific Advisory Board meeting.

8. Score of participants for individual samples

Lab no	Sample A			Sample B			Sample C		
	A	**I**	**T**	**A**	**I**	**T**	**A**	**I**	**T**
1	2	2	**4**	2	2	**4**	2	2	**4**
2	2	2	**4**	2	2	**4**	2	2	**4**
3	2	2	**4**	2	2	**4**	2	2	**4**
4	2	2	**4**	1	2	**3**	2	1	**3**
5	2	2	**4**	2	2	**4**	1	0	**1**
6	2	2	**4**	2	2	**4**	2	2	**4**
7	2	2	**4**	1	1	**2**	2	2	**4**
8	2	2	**4**	2	2	**4**	2	2	**4**
9	2	2	**4**	2	2	**4**	2	2	**4**
10	2	2	**4**	1	1	**2**	2	2	**4**
11	2	2	**4**	2	2	**4**	2	2	**4**
12	2	2	**4**	2	2	**4**	2	2	**4**
13	2	2	**4**	1	1	**2**	2	2	**4**
14	2	2	**4**	0	0	**0**	2	2	**4**
15	2	2	**4**	2	2	**4**	2	1	**3**
16	2	2	**4**	1	1	**2**	2	2	**4**
17	0	0	**0**	0	1	**1**	1	0	**1**
18	2	2	**4**	2	2	**4**	2	1	**3**
19	2	2	**4**	2	2	**4**	2	2	**4**
20	2	2	**4**	2	2	**4**	2	2	**4**
Lab no	**Sample D**			**Sample E**			**Sample F**		
	A	**I**	**T**	**A**	**I**	**T**	**A**	**I**	**T**
1	2	2	**4**	2	2	**4**	2	2	**4**
2	2	1	**3**	2	1	**3**	2	2	**4**
3	2	2	**4**	2	2	**4**	2	2	**4**
4	2	2	**4**	1	2	**3**	2	2	**4**
5	2	2	**4**	2	2	**4**	2	2	**4**
6	2	2	**4**	2	2	**4**	2	2	**4**
7	2	2	**4**	2	2	**4**	2	2	**4**
8	2	2	**4**	2	2	**4**	2	2	**4**
9	2	2	**4**	2	2	**4**	2	2	**4**
10	2	2	**4**	2	2	**4**	2	2	**4**
11	2	2	**4**	2	2	**4**	2	2	**4**
12	2	2	**4**	2	1	**3**	2	2	**4**
13	0	0	**0**	1	1	**2**	2	2	**4**
14	2	0	**2**	2	2	**4**	2	2	**4**
15	2	1	**3**	2	1	**3**	2	2	**4**
16	2	2	**4**	2	2	**4**	2	2	**4**
17	2	2	**4**	0	0	**0**	1	0	**1**
18	2	1	**3**	2	2	**4**	2	2	**4**
19	2	1	**3**	2	1	**3**	2	2	**4**
20	2	2	**4**	2	2	**4**	2	2	**4**

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

9. Total score of participants for individual surveys and their performance in 2015

Lab no	Survey 2015/1 [points]	Survey 2015/2 [points]	Total point 2015
1	12	12	**24**
2	12	10	**22**
3	12	12	**24**
4	10	11	**21**
5	9	12	**21**
6	12	12	**24**
7	10	12	**22**
8	12	12	**24**
9	12	12	**24**
10	10	12	**22**
11	12	12	**24**
12	12	11	**23**
13	10	6	**16***
14	8	10	**18**
15	11	10	**21**
16	10	12	**22**
17	2	5	**7***
18	11	11	**22**
19	12	10	**22**
20	12	12	**24**

* critical error assigned to participant

10. Score summary in 2015

Sample	Diagnosis	Analytical [%]	Interpretatative and recommendations [%]	Total [%]	Number of critical errors
A	***Argininosuccinic aciduria***	95	95	95	1
B	***Sialidosis***	78	83	80	0
C	***Cystinuria***	95	83	89	0
D	***CBS deficiency***	95	80	88	1
E	***MPS IIIC***	90	83	86	0
F	***Propionic acidemia***	98	95	96	0

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

11. Satisfactory performance

The participants who obtained more than 14 points within the calendar year and did not received "critical error" scoring are considered to be performing satisfactory. Eighteen laboratories returning the results achieved a satisfactory performance of more than 14 points without critical error. In 2 instances a serious mistake considered as a critical error has been observed in a total of two participating laboratories (1 laboratory that achieved more than 14 points and 1 laboratory did not reach this threshold). Participants not achieving satisfactory performance will obtain a Performance Support letter in due course.

12. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Czech Republic took place during the ERNDIM Meeting 2015 in Lyon on 1st September 2015, nine laboratories were represented. The following items were discussed during the annual meeting of our DPT centre:

1. Information
   - ERNDIM is aiming at accrediting its activities
   - VK informed participants about news from Executive Committee and SAB
2. Tests required for to 2016
   - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
3. Discussion of results of samples A-F
   - scoring of 2015 results proposed by DPTC Czech Republic organizers has been subsequently evaluated by a second reviewer from an independent DPT center
   - Analytical difficulties in 2015 surveys
     - Analysis of OLS/ sialylOLS in urine
     - Fractionation of urinary GAGs

13. Tentative schedule of DPT scheme and fee in 2016

Sample distribution	February 1, Monday
Start of analysis of Survey 2016/1	February 22, Monday
Survey 2016/1 – results submission	March 14, Monday
Survey 2016/1 – report	May 23, Monday
Start of analysis of Survey 2016/2	May 23, Monday
Survey 2016/2 – results submission	June 13, Monday
Survey 2016/2 – report	August 15, Monday
Annual meeting of participants	September 06, Tuesday
Annual report 2016	April 2017

The annual meeting of participants will take place on September 6th during the SSIEM Annual Symposium in Rome, Italy.

The Executive Board and Board of Trustees of ERNDIM determined the DPT fee for 2016 in the amount of 396 €.

14. Certificate of participation and performance in Proficiency Testing for 2015

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

Prague, March 31, 2016

Prof. Viktor Kožich, MD, PhD
Scientific Advisor to the Scheme
viktor.kozich@vfn.cz

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