

ERNDIM DPT Center Czech Republic

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Proficiency Testing Centre Czech Republic Annual Report 2017

1. Introduction

In 2017 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 2017, for details see the below table:

Country	Number		
	of participants		
Austria	1		
Croatia	1		
Cyprus	1		
Czech Republic	1		
Denmark	1		
Finland	1		
Germany	6		
Latvia	1		
Lithuania	1		
Malaysia	1		
Poland	1		
Portugal	1		
Slovakia	2		
UK	1		
in total	20		

3. Logistics of the scheme

✓ Two surveys: 2017/1 – samples A, B and C 2017/2 – samples D, E and F

Origin of samples: Six urines were obtained from patients with known diagnoses. The common sample was from the DPTC UK (distributed in all five DPT schemes). Other samples were obtained from our repository or were acquired with help of VKS (the Dutch patient organization) and provided by the DPTC Netherlands.

✓ In 2017 the samples have been heat-treated and except for the common sample A were reanalyzed before distribution to participants after the receipt of control shipment from CSCQ.

- In all five samples the typical metabolic profiles were preserved after undergoing this treatment.
- ✓ The samples for Diagnostic Proficiency Testing scheme were distributed via CSCQ in Geneva. On 6th February 2017 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 used: Heat urine to 56 °C for one hour in water bath, make sure that this temperature is achieved in the entire urine sample and not only in the water bath. The urinary samples must be frozen until shipment.
- ✓ Tests required in 2017: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2017

Sample distribution	February 6, Monday
Start of analysis of Survey 2017/1	February 20, Monday
Survey 2017/1 – results submission	March 13, Monday
Survey 2017/1 – report	May 29, Monday
Start of analysis of Survey 2017/2	May 22, Monday
Survey 2017/2 – results submission	June 12, Monday
Survey 2017/2 – report	August 21, Monday
Annual meeting of participants	November 21, Tuesday
Annual report 2017	December 2017

5. Submission of results

	2017/1	2017/2
in time	20	20

6. Samples

Sample A (common sample)

Clinical picture provided with the sample: Infant presented at Emergency Department. Febrile, query infection. Sample collected after commencing therapy.

The common sample was obtained from a 1-month old boy with citrullinemia type 1. The diagnosis was based on demonstrating the urinary excretion of citrulline and orotic acid and very high serum citrulline concentration.

Analytical performance: All participants analyzed amino acids and all participants observed the increased excretion of citrulline; such analytical finding was considered correct and scored by 1 point. All participants also detected elevated excretion of orotic acid, such analytical finding was also considered correct and scored by 1 point. The analytical performance was excellent (100%).

Interpretative proficiency and recommendation: The diagnosis of citrullinemia type 1 was considered correct while suspicion for other urea cycle disorder was considered helpful but incomplete. Confirmation of diagnosis by enzyme analysis using cultured fibroblasts or mutation analysis of *ASS1* gene was considered helpful. The proficiency score for this sample was very good (98%).

Critical error: No critical error for this sample.

Overall impression: Easy DPT sample with very good proficiency score.

Sample B

Clinical picture provided with the sample: A 6 months old girl with kidney stones. The urine sample was collected at age 11 years when the patient was receiving specific treatment.

The sample was obtained from a girl with hyperoxaluria type I, diagnosis was confirmed by enzymatic analysis.

Analytical performance: All participants analyzed organic acids. This was a challenging sample, since oxalate was borderline (only 10 of 20 labs reported elevated excretion of oxalate). Increased excretion of oxalate was considered correct and scored by 1 point. However, excretion of glycolate was clearly elevated and 16 labs reported elevated glycolate excretion, which was scored by 1 point. The analytical performance for this sample was suboptimal (78%).

Interpretative proficiency and recommendation: The diagnosis of hyperoxaluria type 1 was considered correct and scored with 2 points. Confirmation of diagnosis by alanine-glyoxylate aminotransferase assay or mutation analysis of *AGXT* gene was considered helpful. The interpretative performance for this sample was satisfactory (80%).

Critical error: No critical error for this sample.

Overall impression: Challenging DPT sample with a suboptimal total proficiency score.

Sample C

Clinical picture provided with the sample This boy was referred at the age of 5 years with mental and speech retardation, and hyperactivity. He had frequent middle-ear and upper respiratory infections. The sample was obtained at the age of 25 years when the patient was receiving a non-specific therapy.

The sample was obtained from a male with aspartylglucosaminuria, diagnosis was confirmed by molecular genetic analysis.

Analytical performance: 18 labs performed amino acids analysis, only 8 of them reported elevated excretion of aspartylglucosamine. 15 labs performed oligosaccharide analysis and 14 labs concluded abnormal profile. 12 labs concluded aspartylglucosaminuria, such analytical result was considered a correct and scored by 2 points. 2 labs concluded profile typical for GM1-gangliosidosis, this result was considered a partially correct and scored by 1 point. The analytical performance was satisfactory (80%).

Interpretative proficiency and recommendation: The diagnosis of aspartylglucosaminuria was considered correct and scored with 2 points. Confirmation of diagnosis by aspartylglucosaminidase assay or mutation analysis of AGA gene was considered helpful. Suspicion for GM1 ganglisidosis was considered helpful but incomplete and scored with 1 point. Recommendation to carry out analysis of oligosaccharide analysis for those participants that did not perform this analysis was considered also helpful. The interpretative performance for this sample was good (83%).

Critical error: The failure to recognize excretion of aspartylglucosamine in OLS and/or amino acids analysis 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: Typical DPT sample with good total proficiency score (81%).

Sample D

Clinical picture provided with the sample: A 2 years old girl who presented with episode of coma. The urine sample was collected at age 19 years when the patient was receiving specific treatment. The sample was obtained from a woman with MCAD deficiency, diagnosis was confirmed by molecular genetic analysis.

Analytical performance: All participants analysed organic acids. Seventeen labs reported increased excretion of glycine conjugates (hexanoylglycine, phenylpropionylglycine, suberylglycine), which was considered correct and scored by 2 points. Since 3 laboratories missed the presence of glycine conjugates the analytical performance for this sample was suboptimal (85%).

Interpretative proficiency and recommendation: The diagnosis of MCAD deficiency was considered correct and scored with 2 points; recommendation to analyse the ACADM gene was considered helpful. Two laboratories that missed glycine conjugates excretion recommended to

carry out analysis of acylcarnitines in dry blood spot; this interpretation was considered helpful and scored with 1 point. The interpretative performance for this sample was good (90%).

Critical error: The failure to recognize abnormal excretion of glycine conjugates without recommending additional acylcarnitine analysis in dry blood spot 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: Typical DPT sample with good total proficiency score (88%).

Sample E

Clinical picture provided with the sample: A 2 years old boy was admitted to hospital following a head trauma. The neurologist reported macrocephaly, facial stigmatization and psychomotor retardation. The sample was collected at the age of 5 years; patient did not receive any therapy.

The sample was obtained from a boy with mucopolysaccharidosis type II due to deficiency of iduronate 2-sulfatase, diagnosis was confirmed by molecular genetic analysis.

Analytical performance: Elevated excretion of glycosaminoglycans (1 point) and an increased proportion of dermatan sulfate (1 point) were considered a correct analytical result. Increased excretion of GAGs or increased proportion of dermatan sulfate only was scored as partially correct. Analytical performance was good (85) %.

Interpretative proficiency and recommendation: The diagnosis of mucopolysaccharidosis type II was considered correct while suspicion for MPS (other types of MPS or non-specified MPS) was considered helpful but incomplete. Confirmation of diagnosis by enzyme assay of iduronate 2-sulfatase activity in fibroblasts/leucocytes and/or mutation analysis of IDS gene were considered helpful. Recommendation to carry out analysis of GAG fractionation for those participants that did not perform this analysis was considered also helpful. The proficiency score for this sample was very good (90%).

Critical error: No critical error for this sample.

Overall impression: Typical DPT sample with good total proficiency score (88%).

Sample F

Clinical picture provided with the sample: This boy was referred at the age of 6 years for dark stain on underwear. The sample was collected at the age of 7 years; patient did not receive any therapy.

The sample was obtained from a boy with alkaptonuria due to deficiency of homogentisate dioxygenase. The diagnosis was solely based on demonstrating grossly increased urinary excretion of homogentisic acids.

Analytical performance: All participants analysed organic acids and observed massive excretion of homogentisic acid, which was considered correct analytical result. Analytical performance was excellent (100%).

Interpretative proficiency and recommendation: The diagnosis of alkaptonuria was considered correct. A plethora of recommendations was reported, all the following were considered correct: massive excretion of homogentisate is sufficient for diagnosis, enzymatic confirmation is not required, mutation analysis of *HGD* gene is available. The proficiency score for this sample was excellent (100%).

Critical error: No critical error for this sample.

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

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.

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

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 2017. Provisional scores assigned by the organizers were reviewed by an independent advisor from another DPT Centre and final scoring was approved by the ERNDIM Scientific Advisory Board on November 23, 2017.

Normal samples are usually not eligible for Critical Error. The main argument is that one cannot be certain that a sample is normal. The patient could, for example, have an IEM that we did not know at the time of analysis, but did result in subtle metabolite abnormalities that most of the participants were not aware of. However, when the sample was obtained from a patient not suspected of having an IEM and the findings reported were not identified by the rest of the participants then this diagnosis could potentially result in treatment that is harmful for the patient and the findings could constitute a critical error. With effect from 2017, the SAB will determine critical errors on a case by case basis.

8. Score of participants for individual samples

Lab	S	ample	A	S	ample l	В	S	ample	C
no	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	0	1	1
4	2	1	3	2	2	4	1	1	2
5	2	2	4	2	0	2	2	2	4
6	2	2	4	0	2	2	2	2	4
7	2	2	4	2	2	4	2	2	4
8	2	2	4	0	0	0	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	0	0	0	2	2	4
13	2	2	4	2	2	4	2	2	4
14	2	2	4	2	2	4	2	2	4
15	2	2	4	2	2	4	2	2	4
16	2	2	4	0	0	0	0	0	0
17	2	2	4	2	2	4	2	2	4
18	2	2	4	2	2	4	2	2	4
19	2	2	4	1	2	3	0	0	0 CE
20	2	2	4	2	2	4	1	1	2

Lab	Sample I		D	S	ample	E	S	ample	F
no	A	I	T	A	I	T	A	I	T
1	2	2	4	2	2	4	2	2	4
2	2	2	4	1	1	2	2	2	4
3	2	2	4	2	2	4	2	2	4
4	0	0	0 CE	2	2	4	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	0	1	1	2	2	4
13	2	2	4	1	1	2	2	2	4
14	2	2	4	2	2	4	2	2	4
15	0	1	1	2	2	4	2	2	4
16	2	2	4	0	1	1	2	2	4
17	2	2	4	2	2	4	2	2	4
18	2	2	4	2	2	4	2	2	4
19	0	1	1	2	2	4	2	2	4
20	2	2	4	2	2	4	2	2	4

A – Analytical score, I – Interpretative score, T – Total score, CE - Critical error

9. Total score of participants for individual surveys and their performance in 2017

Lab	Survey 2017/1	Survey 2017/2	Total point
no	[points]	[points]	2017
1	12	12	24
2	12	10	22
3	9	12	21
4	9	8	17 CE
5	10	12	22
6	10	12	22
7	12	12	24
8	8	12	20
9	12	12	24
10	12	12	24
11	12	12	24
12	8	9	17
13	12	10	22
14	12	12	24
15	12	9	21
16	4	9	13
17	12	12	24
18	12	12	24
19	7	9	16 CE
20	10	12	22

CE - Critical error assigned to the participant

10. Score summary in 2017

Sample	Diagnosis	Analytical [%]	Interpretatative and recommendations [%]	Total [%]	Number of critical errors
A	Citrullinemia type I	100	98	99	0
В	Hyperoxaluria type 1	78	80	79	0
C	Aspartylglucosaminuria	80	83	81	1
D	MCAD deficiency	85	90	88	1
E	MPS II	85	90	88	0
F	Alkaptonuria	100	100	100	0

[&]quot;Easy" and "difficult" samples were included in the surveys. The analytical performance was good for 5 samples; however, there were 2 critical errors due to analytical mistakes. The interpretative performance was adequate for samples with adequate results of analytical investigations.

11. Satisfactory performance

Performance of the participant that obtained 15 points and more within the calendar year and that did not receive "critical error" mark is considered satisfactory. Seventeen laboratories returning the results achieved a satisfactory performance. A serious mistake considered as a critical error has been observed in a total of two participating laboratories (although these laboratories achieved 15 points and more and would otherwise be considered as having adequate performance). One laboratory achieved less than 15 points. 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 2017 in Manchester on 21st November 2017, 10 participants from 7 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
- 2. Tests required for to 2018
 - amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines
- 3. Discussion of results of samples A-F
 - scoring of 2017 results proposed by DPTC Czech Republic organizers has been subsequently evaluated by a second reviewer from an independent DPT center
 - Analytical difficulties in 2017 surveys
 - sample B: Routine organic acid analysis with liquid extraction followed by gas chromatographic analysis may fail to detect increased concentrations of oxalate and glycolate. Appropriate reference ranges are also an important factor for correct diagnosis.
 - sample C: Oligosaccharides profile with orcinol staining in the urine of patient suffering from aspartylglucosaminuria may have resembled GM1 gangliosidosis. Recommendation to perform additional staining in samples with abnormal OLS profile with orcinol staining was proposed.
 - Critical error in aspartylglucosaminuria: Participants agreed that failure to recognize aspartylglucosaminuria should be considered a critical error. Although the disease is at

present not treatable, correct diagnosis is necessary for genetic counseling in the family and possible prenatal diagnosis.

13. Tentative schedule of DPT scheme and fee in 2018

Sample distribution	February 5, Monday
Start of analysis of Survey 2018/1	February 26, Monday
Survey 2018/1 – results submission	March 19, Monday
Survey 2018/1 – report	May 28, Monday
Start of analysis of Survey 2017/2	May 28, Monday
Survey 2018/2 – results submission	June 18, Monday
Survey 2018/2 – report	August 20, Monday
Annual meeting of participants at SSIEM	September 4, Tuesday
Annual report 2018	December 2018

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

The Executive Board and Board of Trustees of ERNDIM determined the DPT fee for 2018 in the amount of 437 €.

14. Certificate of participation and performance in Proficiency Testing for 2017 Results of DPT Scheme will be included in the Certificate of participation and performance, which will be issued by ERNDIM.

Prague, December 1, 2017

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