ERNDIM DPT Center Basel

University Children's Hospital
Metabolic Unit

Roemergasse 8, 4005 Basel, Switzerland
phone: +41 61 685 62 75 or 61 685 63 23
fax: +41 61 685 60 16

Proficiency Testing Centre Basel
Annual Report 2006

1. Introduction

In 2006 proficiency testing in our centre was run as a regular ERNDIM scheme.

2. Geographical distribution of participants

Twenty laboratories from 10 countries have participated in our Diagnostic Proficiency Testing scheme in 2006, for details see the below table:

Country	Number of participants
Austria	1
Canada	2
Czech Republic	1
Germany	6
Israel	1
Norway	1
Sweden	1
Switzerland	2
UK	1
USA	4
total	**20**

3. Logistics of the scheme

- Two surveys: **2006/1** – samples A, B and C / **2006/2** – samples D, E and F
- Origin of samples: Four urines were obtained from patients with known diagnoses and one sample was obtained from a patient without any known IEM (samples were provided by the organizer) and the common sample from our Dutch colleagues was distributed in all 5 DPT schemes. All samples were analyzed in our lab after heat-treatment, diagnostically relevant metabolites were detected in all six samples after 3-day incubation at RT mimicking possible changes during transport.
- Six heat-treated urines together with results protocols were shipped at once to the participants at ambient temperature using the TNT courier service. Eleven parcels were received within 5 days, five within 7 – 8 days, the receiving date was not indicated for three parcels and one parcel was reported to have been received one day before shipment!
- The following protocol for heat inactivation was used: 1. Add thiomersal 100 mg/l of urine; 2. Heat urine to 56°C for one hour in water bath. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. The urinary samples have to be frozen until shipment
- Tests required in 2006: amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/pyrimidines

4. Schedule of the scheme in 2006

Sample distribution	April 26, Wednesday
Start of analysis of Survey 2006/1	May 4, Thursday
Survey 2006/1 – results submission	May 24, Wednesday
Survey 2006/1 – evaluation by organisers (report)	June 16, Friday
Start of analysis of Survey 2006/2	June 26, Monday
Survey 2006/2 – results submission	July 17, Monday
Survey 2006/2 – evaluation by organisers (report)	Sept 1, Friday
Annual meeting of the participants	October 5, Thursday
Annual report 2006	December

5. Receipt of samples and results

Date of receipt of samples (samples sent on April 26, 2006)

Date of receipt (reported by participants)	**Number of participants**
1 day	6
2 days	2
5 days	3
6 days	3
7 days	2
not indicated	3
25 April	1

Deadlines of results submission

	2006/1	**2006/2**
Deadline or before	19	15
8 days delay	1	-
14 days delay	-	1
17 days delay	-	1
32 days delay	-	1
no results		2

6. Scoring system

Analytical performance, interpretative proficiency and recommendations for further investigations are evaluated. Due to the large variability in reporting results in various countries recommendations pertaining 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** is 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. The scores were calculated only for laboratories submitting results.

7. Results of samples and evaluation of reporting

Sample A

Patient: The sample was obtained from a 17-year old boy with argininosuccinic aciduria assumed to be due to argininosuccinate lyase deficiency and cared for in Basel. The diagnosis was based on examination of urine and plasma amino acids as well as the periodical hyperammonaemia. Enzymatic or mutational analysis has not been performed but the plasma and urine amino acid profiles and clinical symptoms leave no doubt as to the diagnosis. The child grew up in Turkey and came to Switzerland without any diagnosis at the age of 15 years. At that time his general nutritional state was poor. B_{12} deficiency was found coincidental to the basic defect.

Analytical performance: 18 Laboratories reported amino acid analyses and 15 were able to correctly identify the metabolites indicative of argininosuccinate lyase deficiency. This was considered as satisfactory performance, anything else as inadequate. One laboratory focussed on B_{12} metabolism but the finding of low B_{12} in this patient was purely coincidental and reflected poor nutrition. Three laboratories reported homocystine, 2 cystathionine and one leucine to be increased, possibly due to miss identification of ASA. Another found increased cystathionine and low methionine (but not homocystine) leading to a putative diagnosis of methionine synthase deficiency. The lack of a correct diagnosis in one quarter of the labs reinforces the importance of reliable amino acid analysis in the diagnosis of IEMs.

The analytical performance of this sample was 75%.

Interpretative proficiency: Diagnosis of argininosuccinic aciduria due to argininosuccinate lyase deficiency was considered correct. The proficiency score of 75% was less than expected for this quite straightforward sample.

Recommendations: In our opinion the most important advice for follow-up investigation included plasma amino acid analysis and confirmation of the defect by enzyme assay and possible mutation analysis. When one of these two follow up measures was given the recommendations were considered helpful.

Quantitative data: **Creatinine:**

0.23; 1.19; 1.80; 1.85; 1.90; 1.9: 1.90; 1.91; 1.99; 2.00; 2.01; 2.09; 2.1; 2.10; 2.12; 2.12; 2.21; 2.21; 2.31; 2.33 (mmol/l), mean: 1.9, median: 2.0.

- **Amino acids:Arginosuccinic acid**: 685; 1380; 2000; 2360; 2513; 2636; 3999; 5202; (mmol/mol creatinine)
  Arginosuccinic acid anhydride I/II: 331; 403 (mmol/mol creatinine)

Sample A amino acids: see attached powerpoint file

Sample B

Patient: This sample came from a 5 month old boy with hepato-splenomegaly, muscular hypotonia and severely delayed psychomotor development. The diagnosis of GM1-gangliosisdosis was first suspected because of the typical pattern of urinary oligosaccharides and ß-galactosidase deficiency was subsequently confirmed in leukocytes.

Analytical performance: The performance of oligosaccharide analysis was considered essential for the diagnosis in this case. The pattern of oligosaccharides on TLC was reported to be abnormal by 12 participants. The analytical performance of this sample was low (60%)

Interpretative proficiency: The pattern of oligosaccharides on TLC is in our opinion characteristic of GM1-gangliosidosis and mention of this diagnosis was considered necessary to obtain two points. Reporting of sialidosis was considered only partially correct. Following discussion at the DPT meeting in Prague this was modified and accepted as correct (Proficiency score: 60%).

Recommendations: Measurement of beta-galactosidase activity is the necessary follow up investigation.

Quantitative data:

- **Creatinine:**
  1.15; 1.2; 1.21; 1.23; 1.23; 1.26; 1.3; 1.33; 1.33; 1.34; 1.4; 1.4; 1.4; 1.41; 1.41; 1.49; 1.5; 1.6; 1.9 (mmol/l), mean: 1.38, median: 1.37.

Sample B TLC – Oligosaccharides see attached powerpoint file

Sample C

Patient: The urine sample was obtained from a 23 year old female who had first presented at the age of 5 years due to episodic vomiting, hypoglycaemia and loss of consciousness. She has remained essentially well on treatment and the urine was collected at the age of 23 whilst assymptomatic. The diagnosis was confirmed by enzyme assay on skin fibroblasts. Treatment had included avoidance of fasting, frequent meals and intermittently carnitine.
Analytical performance: Nineteen laboratories analysed organic acids and correctly identified one or more key acyl glycines leading to the correct diagnosis of MCAD deficiency. The remaining laboratory did analyse organic acids but reported a normal finding. The analytical performance of this sample was high (95%)
Interpretative proficiency: The diagnosis of medium chain acylCoA dehydrogenase deficiency was considered correct. 19 labs concluded the correct diagnosis so that the interpretative proficiency for this sample was excellent (95%).
Recommendations: follow up by measuring acyl carnitines in blood and or enzyme assay and or mutation analysis was considered correct.

Quantitative data:

- **Creatinine:**
  0.52; 5.21; 5.3; 5.39; 5.42; 5.44; 5.48; 5.5; 5.6; 5.64; 5.68; 5.75; 5.89; 5.9; 6.07; 6.19; 6.2; 6.4; 6.45; 6.8 (mmol/l), mean 5.54, median 5.66.
- **Organic Acids:**
  Hexanoylglycine:
  3.3; 9; 27; 31; 35; 42; 117; (mmol/mol creatinine); 39.03 (µg/mg creatinine)
  Phenylproprionylglycine:
  5; 7; 17 (mmol/mol creatinine); 21.42 (µg/mg creatinine)
  Suberylglycine:
  6; 27; 31; 40; 42; 80; 262; (mmol/mol creatinine); 93.76 (µg/mg creatinine)

Sample D
Patient: The sample was obtained at 3 ½ years of age from a now 5½ year old boy with dihydropyrimidine dehydrogenase deficiency. The diagnosis was based on examination of urine, plasma and CSF organic acids. The diagnosis was confirmed at the AMC Amsterdam by enzyme assay and mutation analysis.
Analytical performance: 18 Laboratories reported organic acid analysis and 16 were able to correctly identify the metabolites indicative of dihydropyrimidine dehydrogenase deficiency. Two laboratories did not report these abnormalities but did make the correct diagnosis by purine and pyrimidine analysis. In all 13 laboratories analysed purines and pyrimidines all reporting abnormal metabolite(s). A correct diagnosis but lack of abnormalities on organic acid analysis was considered as only partially satisfactory performance.
The analytical performance of this sample was 94%.
Interpretative proficiency: Diagnosis of thyminuria / uraciluria due to dihydropyrimidine dehydrogenase deficiency was considered correct. The proficiency score of 97% was very satisfactory.
Recommendations: In our opinion the most important advice for follow-up investigation was confirmation of the defect by enzyme assay and possible mutation analysis. When at least one of these two follow up measures was given the recommendations were considered helpful.
Overall impression: the total overall score of 93% indicates very satisfactory performance for this defect.

Quantitative data:

- **Creatinine:** 2.65; 2.74; 2.82; 2.99; 3.10; 3.2; 3.2; 3.3; 3.36; 3.4; 3.49; 3.54; 3.66; 3.7; 3.7; 3.84; 4.0 (mmol/l), mean 3.3, median 3.4.
- **Organic Acids**:
  Uracil: 101; 241; 600; 612; 722; 847; (mmol/mol creatinine)
  Thymine: 184.3; 393; 461; 666; 1100; (mmol/mol creatinine)
- **Purines/Pyrimidines**:

Uracil: 0.857; 241; 280; 396; 629; 643; 657; 815; 1631; 1631 (mmol/mol creatinine)
Thymine: 0.659; 11; 11; 220; 334; 391; 393; 438; 525; 486.6; 639 (mmol/mol creatinine)

Sample D GC-MS: see attached powerpoint file

Sample E

Patient: This sample came from an 8 ½ year old boy who was admitted with a history of recurrent infections, mild mental retardation and he had been operated on previously for a congenital heart defect. At this time he suffered from an infection of the upper airways and was treated with the antibiotic augmentin, a penicillin derivative.

Analytical performance: The relatively unspecific findings justify screening for all analyte groups, in particular amino acid and organic acid analysis should be performed. No specific abnormalities should have been found even though several metabolites derived from the antibiotic treatment can be detected on amino acid and organic acid analysis. All laboratories performed amino acid analysis and organic acid analysis. 13 laboratories reported no evidence of an inborn error some mentioning minor amino acid abnormalities. One point each was given for normal amino acids and organic acids. Mention of specific amino acid or organic acid abnormalities was considered unsatisfactory performance. The analytical performance of this sample of 86% was fairly satisfactory.

Interpretative proficiency: This urine shows the presence of several ninhydrin positive substances derived from the antibiotic treatment. Some of these may be falsely identified as specific amino acids but do not suggest a specific disorder. The pattern due to penicillin derived antibiotics should be recognisable. No inborn error is the correct interpretation. Proficiency score: 75%.

Recommendations: Since in our opinion it should be possible to recognise the fairly common occurrence of antibiotic interference, a recommendation for no follow up tests is correct. This was modified after discussion at the DPT participants meeting in Prague to accept clinically relevant tests based on correct analytical findings as correct.

Overall impression: the only partially satisfactory total overall score of 78%, with a relatively low score for recommendations, reflects the misinterpretation of peaks due to the antibiotic treatment as specific amino acids as well as a tendency towards, in our opinion, unnecessary recommendations for further tests.

Quantitative data:

- ***Creatinine:***
  4.25; 5.2; 5.29; 5.35; 5.55; 5.55; 5.66; 5.7; 5.81; 5.88; 6.0; 6.04; 6.06; 6.1; 6.36; 6.5; 7.0 (mmol/l), mean: 5.8, median: 5.8

Sample E, Ion exchange chromatography of amino acids & High-voltage electrophoresis of amino acids: see attached powerpoint file

Sample F (common sample)
Patient: The sample was obtained from a 24 year old female patient with the adult form of hypophosphatasia. The diagnosis had been confirmed by demonstrating deficiency of alkaline phosphatase in leukocytes (13 nmol/mg.hr; reference range, 100-5000).
Analytical performance: Eighteen participants performed amino acid analysis and twelve reported elevated excretion of the critical metabolite phosphoethanolamine. This was considered to be the correct analytical finding. The analytical performance for this sample was 67%.
Interpretative proficiency: The diagnosis of hypophosphatasia due to deficiency of tissues-non-specific (liver/bone/kidney) alkaline phosphatase isoenzyme (TNSALP) was considered correct. Twelve participants reached the correct diagnosis. Interpretative proficiency for this sample was only moderately satisfactory (67%).
Recommendations: The recommendation to measure alkaline phosphatase activity in serum/plasma was considered satisfactory. In addition measurement of ALP isoenzyme levels or TNSALP isoforms in serum can be helpful. Measurement of pyrophosphate and pyridoxal-5´-phosphate levels can be important in unclear cases.
Overall impression: Almost one third of laboratories failed to make the diagnosis probably reflecting the relatively mild form of the disease as well as possible analytical difficulties with compounds which elute early in the ion-exchange method.

Quantitative data:

- **Creatinine:**
  1.1; 1.42; 1.67; 1.74; 1.8; 1.8; 1.8; 1.8; 1.8; 1.84; 1.98; 1.99, 2.0, 2.0; 2.1; 2.12; 2.2 (mmol/l), mean.1.8, median: 1.8.

- **Amino Acids:**
  Phosphoethanolamine: 109; 114; 120; 122; 129.3; 140; 151; 156; 187.3; 243; 259 (mmol/mol creatinine); 830 (µmol/g creatinine).

Sample F ion exchange chromatography of amino acids: see attached powerpoint file

8. Score of participants for individual samples

Survey 2006/1

Lab no	Sample A Arginiosuccinic acid				Sample B GM1- gangliosidosis				Sample C MCADI			
	A	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**
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	0	0	0	**0**	0	0	0	**2**	2	2	1	**5**
4	2	2	1	**5**	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	1	**5**	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**	0	0	1	**1**
9	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
10	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
11	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
12	2	2	1	**5**	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**	2	2	1	**5**	2	2	1	**5**
15	0	0	0	**0**	0	0	1	**1**	2	2	1	**5**
16	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
17	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
18	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
19	0	0	0	**0**	0	0	0	**0**	2	2	1	**5**
20	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
	75%	75%	75%	**75%**	60%	60%	80%	**66%**	95%	95%	100%	**96%**

Survey 2006/2

Lab no	Sample D DiHydropyrimidine DeH def				Sample E No disorder antibiotics				Sample F Hypophosphatasia			
	A	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**
1	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
2	2	2	1	**5**	2	2	0	**4**	0	0	0	**0**
3	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**
4	2	2	0	**4**	2	2	1	**5**	2	2	1	**5**
5	1	2	1	**4**	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**	2	2	1	**5**	2	2	1	**5**
8	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
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	2	2	1	**5**	1	0	0	**1**	0	0	0	**0**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	1	0	0	**1**	0	0	0	**0**
15	1	1	0	**2**	1	0	0	**1**	2	2	1	**5**
16	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
17	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
18	2	2	0	**4**	2	1	0	**3**	0	0	0	**0**
19	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
20	2	2	1	**5**	2	2	1	**5**	0	0	1	**1**
	94%	97%	83%	**93%**	86%	75%	67%	**78%**	67%	67%	72%	**68%**

A - Analytical score. I – Interpretative score, R – Recommendations.

Survey 2006/1 – Score summary

Sample	Diagnosis	Analytical [%]	Interpreta-tative [%]	Recommen-dations [%]	Total [%]
A	**Argininosuccinic aciduria**	75%	75%	75%	75%
B	**GM1-gangliosidosis**	60%	60%	80%	66%
C	**MCAD deficiency**	95%	95%	100%	96%
D	**Dihydropyrimidine dehydrogenase deficiency**	94%	97%	83%	93%
E	**No evidence of an IEM**	86%	75%	67%	78%
F	**Hypophosphatasia**	67%	67%	72%	68%

9 Total score of participants for individual surveys and their performance in 2006

Lab no	Survey 2006/1 (points)	Survey 2006/2 (points)	Total points 2006
1	15	14	**29**
2	15	9	**24**
3	7	11	**18**
4	15	14	**29**
5	15	14	**29**
6	15	15	**30**
7	11	15	**26**
8	11	0	**11**
9	10	15	**25**
10	15	15	**30**
11	11	6	**17**
12	15	15	**30**
13	15	15	**30**
14	10	6	**16**
15	6	8	**14**
16	10	0	**10**
17	11	10	**21**
18	10	7	**17**
19	5	15	**20**
20	15	11	**26**

10. Assessment of performance

Steps are being taken within the Scientific Advisory Board of ERNDIM to set the level of good performance within a proficiency scheme. Until this has been achieved it is has been decided to issue letters of support to those laboratories with clear poor performance taken as a score of less than 15 points (>50%) within the calendar year. Two laboratories failed to submit results for the second circulation. Of the remainder just one lab scored less than 15 points.

11. Annual meeting of the participants

The annual meeting of participants of the Proficiency Testing Centre Prague took place during ERNDIM/Eurogentest meeting in Prague on 5th October 2006

12. Changes planned for 2007

A system for submission and evaluation of results and reporting via internet is now being developed by B. Fowler, P. Litynski and V. Kozich. It is hoped to be able to introduce this system on a pilot scale to allow testing by participants from the Basel and Prague centres. Participants will be notified of developments in due course.

Tentative schedule of DPT scheme and fee in 2007

Sample distribution	April 25, Wednesday
Start of analysis of Survey 2007/1	May 7, Monday
Survey 2007/1 – results submission	May 28, Monday
Survey 2007/1 – report	June 18, Monday
Start of analysis of Survey 2007/2	June 25, Monday
Survey 2007/2 – results submission	July 16, Monday
Survey 2007/2 – report	August 10, Monday
Annual meeting of participants	September 4, Tuesday
Annual report 2007	December

The next annual meeting of participants will take place on September 4th during the 43rd SSIEM Annual Symposium in Hamburg.

The Executive Board of ERNDIM determined the fee for 2007 in the amount of 284 €.

Certificate of participation in Proficiency Testing for 2006

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

Basel, February 12, 2007

Prof. Brian Fowler
Scientific Advisor to the Scheme
e-mail: Brian.Fowler@unibas.ch

Marianne Zaugg

Piotr Litynski