ERNDIM DPT Centre Basel

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Diagnostic Proficiency Testing

Survey 2008

Annual Report

prepared by B. Fowler, K. Honegger, M. Zaugg,
November 2008

1. Geographical distribution of participants

This year, 23 laboratories from 10 countries have participated in our scheme:

Country	Number of participants
Austria	1
Canada	3
Estonia	1
Germany	6
Israel	1
Norway	1
Sweden	2
Switzerland	2
UK	1
USA	5

2. Samples

The samples contain a small amount of thimerosal and have been heat-treated. They were pre-analysed in our institute after 3 days incubation at ambient temperature (to mimic possible changes that might arise during transport). In all six samples the typical metabolic profiles were preserved after this process.

3. Shipment of the samples

The urinary samples were distributed to participants on April 21 at ambient temperature using the courier TNT Swiss Post.
Delivery of samples took between 1 and 2 days according to the tracking by the courier, however, the delivery times stated by the participants varied between 1 to 7 days. Nineteen participants returned their results by the deadline, 3 with a short delay and 1 was 16 days late due to communication problems. Regardless of the delay all reported results were accepted by the organisers.

4. Tests

Analyses of amino acids, organic acids, mucopolysaccharides and purines/pyrimidines were required in 2008.

5. Schedule of the scheme in 2008

Task	Due
Sample distribution	April 21, Monday
Start of analysis of Survey 2008/1	May 5, Monday
Survey 2008/1 - Results submission	May 26, Monday
Survey 2008/1 - Reports	June 16, Monday
Start of analysis of Survey 2008/2	June 23, Monday
Survey 200/2 – Results submission	July 14, Monday
Survey 2008/2 - Reports	August 8, Friday
Annual meeting of participants	September 2, Tuesday
Annual Report 2008	November

6. Receipt of samples and results

Date of receipt of samples (sent on April 21, 2008)

Receipt (days after shipment)	Receipt (reported by participants)	Delivery (by TNT Swiss Post)
1 day	7 participants	13
2 days	8	10
3 days	4	-
7 days	1	-
no information	3	-

Date of reporting of results

Receipt of results	Part 1 (deadline May 26)	Part 2 (deadline July 14)
deadline or before	19 participants	18
1 day delay	1	5
2 days delay	1	-
7 days delay	1	-
16 days delay	1	-

7. Scoring system

Three criteria are evaluated: analytical performance, interpretative proficiency and recommendations for further investigations. 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 summarised by the scheme organisers.

A	Analytical performance	Correct results of the appropriate tests	2	max 2
		Partially correct or non-standard methods	1	
		Unsatisfactory or misleading	0	
I	Interpretative proficiency	Good (diagnosis was established)	2	max 2
		Helpful but incomplete	1	
		Misleading/wrong diagnosis	0	
R	Recommendations	Helpful	1	max 1
		Unsatisfactory or misleading	0	

The **total score** is calculated as a sum of these three criteria. The maximum to be achieved is 5 points per sample. The scores were calculated only for laboratories submitting results.

8. Results of samples and evaluation of reporting

Sample A: Aminoacylase 1 deficiency

Patient: the sample was obtained from a 11 year old boy with aminoacylase 1 deficiency who was receiving no treatment. The diagnosis was based on urine organic acid analysis and was confirmed by the finding of mutations in the *ACY1* gene. This sample was contributed by Dr. J. O. Sass, Freiburg, Germany.

Analytical performance: 22 laboratories reported organic acid analyses, but only 8 (1 in a cluster laboratory) were able to correctly identify a number of N-acetylated amino acids which scored 2 points. The analytical performance of this sample was only 35%.

Interpretative proficiency: diagnosis of aminoacylase 1 deficiency was considered correct. The proficiency score was 35%.

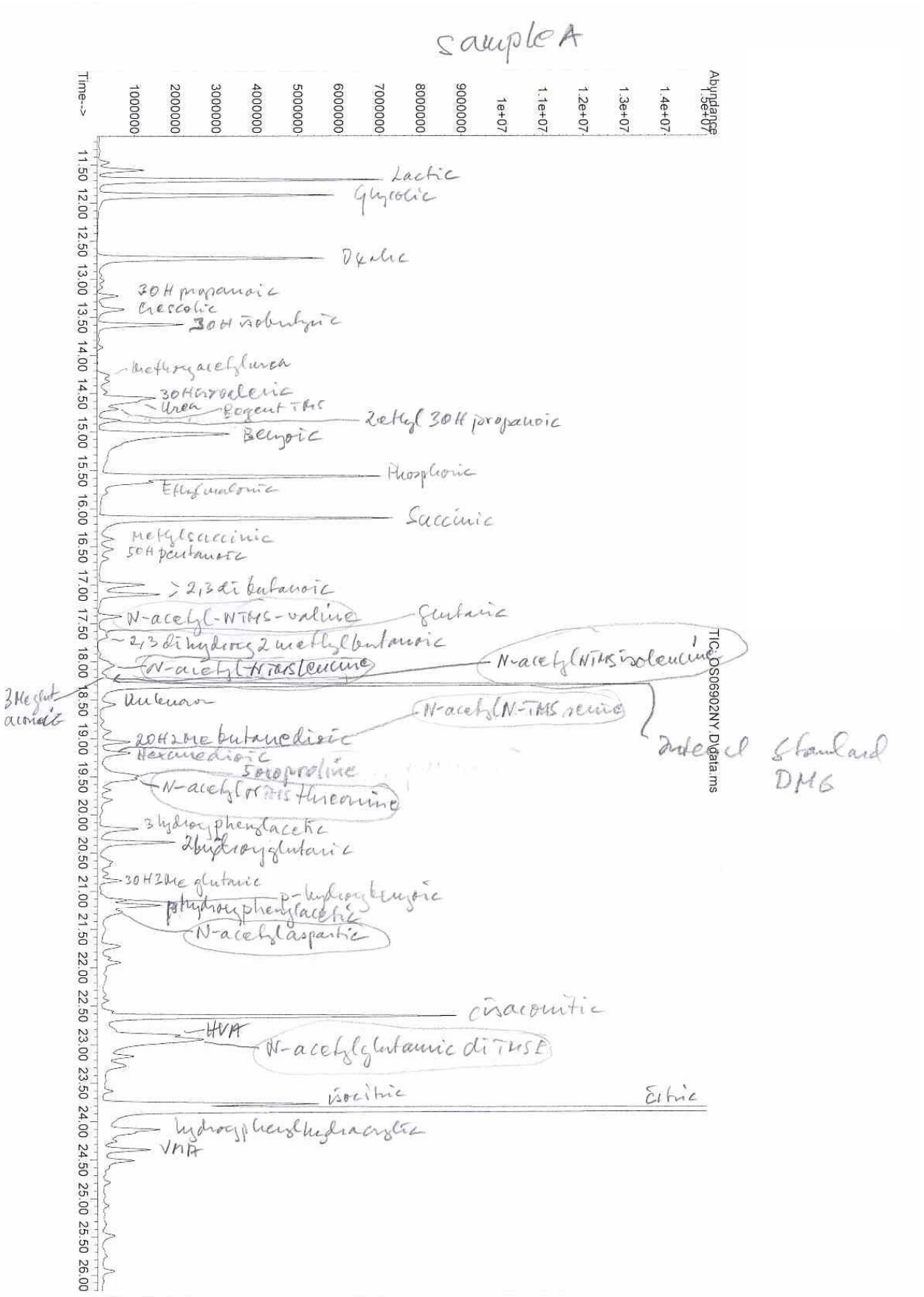
Recommendations: we consider follow-up by enzyme assay (aminoacylase 1) and/or mutation analysis (*ACY1* gene) as important.

Overall impression: the low overall performance, with only 8 laboratories making a correct diagnosis, may reflect different extraction efficiencies of N-acetylated amino acids as well as unfamiliarity with the metabolite profile in this new disorder.

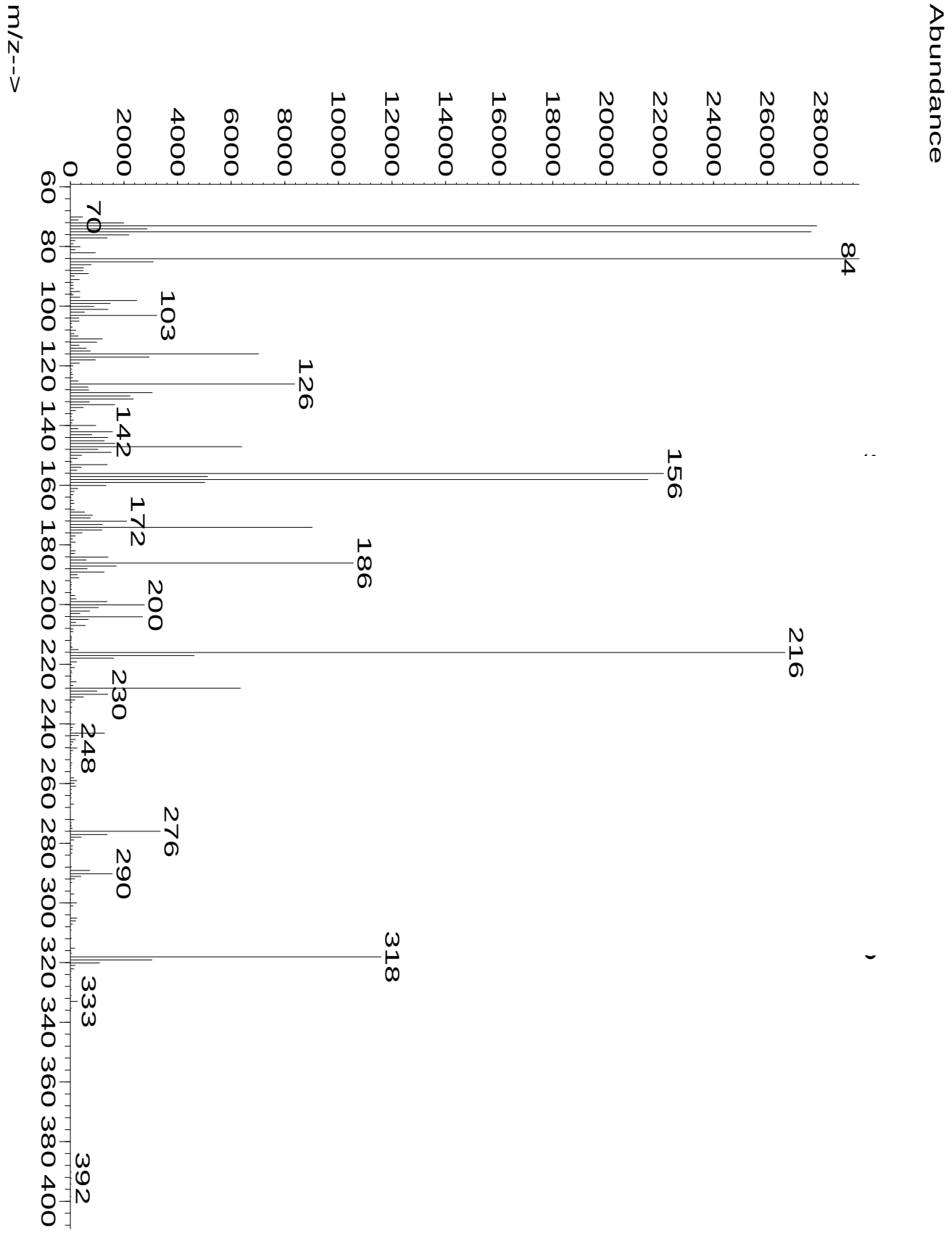
Quantitative data:
Creatinine: mean 10.3, median 10.4, range 8.9 – 11.8

N-acetylated amino acids:	N-acetyl-Glutamic acid
	N-acetyl-Valine
	N-acetyl-Leucine
	N-acetyl-Isoleucine
	N-acetyl-Methionine
	N-acetyl-Glycine
	N-acetyl-Alanine
	N-acetyl-Serine
	N-acetyl-Threonine
	N-acetyl-Aspartic acid

Organic acid chromatogram of sample A



Mass spectrum of N-Acetyl-glutamic acid diTMS, sample A



Sample B: Adenylosuccinate lyase deficiency (ADSL)

Patient: this sample came from a 11 months old girl with untreated ADSL (adenylosuccinate lyase deficiency). The sample was provided by Dr. B. Woldseth, Oslo, Norway. The diagnosis had been confirmed by the finding of two mutations n the *ADSL* gene (Prof. G. Van den Berghe).

Analytical performance: purine/pyrimidine analysis was considered essential for the diagnosis in this case and was performed by 12 laboratories. Eight labs reported increased excretion of succinyladenosine and/or SAICAR and this was considered correct and received 2 points. One lab reported unknown purine/pyrimidine peaks and received 1 point. Analytical performance was 37%.

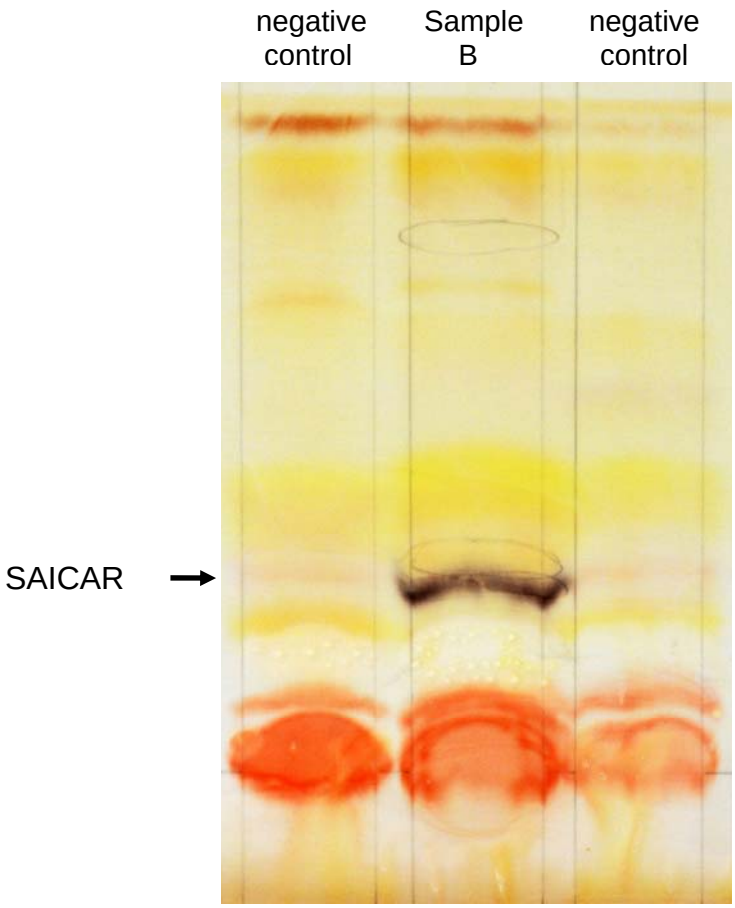
Interpretative proficiency: the diagnosis of ADSL is considered correct and received 2 points. 1 point was given for indication of a purine/pyrimidine disorder. Proficiency score was 37%.

Recommendations: confirmation of diagnosis by enzyme assay (adenylosuccinase), mutation analysis (*ADSL* gene), amino acid (Asp, Gly) analysis after acid hydrolysis and determination of the succinyladenosine/SAICAR ratio were considered helpful.

Overall impression: this difficult sample with only 9 laboratories detecting the abnormality indicates deficits in purine/pyrimidine screening.

Quantitative data:
Creatinine: mean 2.0, median 2.0, range 1.4 – 2.3

SAICAR TLC of sample B



Sample C: Mucopolysaccharidosis type VI (Maroteaux-Lamy)

Patient: this sample was obtained from a 8 year old girl suffering from mucopolysaccharidosis type VI (Maroteaux-Lamy). Enzyme replacement treatment had been begun in the patient shortly before the urine was collected. This urine was provided by Dr. M. Baumgartner, Zurich, Switzerland. The diagnosis had been confirmed by the finding of deficient N-acetyl-galactosamine-4-sulphatase in leucocytes.

Analytical performance*:* mucopolysaccharide analysis was considered essential. The finding of increased GAG and dermatan sulphate was considered correct. 22 laboratories performed mucopolysaccharide analysis. 20 found increased GAG which received 1 point. 1 additional point was given for the finding of DS. The superfluous findings of mild changes in amino acid levels were reported by some labs. The analytical performance of this sample was 70%.

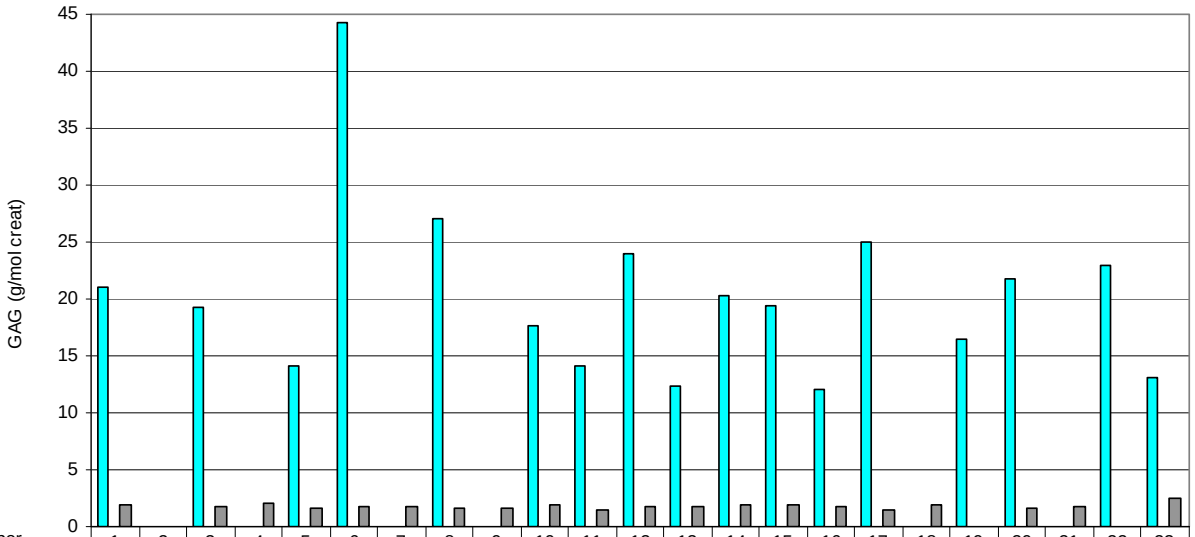
Interpretative proficiency: a diagnosis of MPS in general received 1 point, and 1 point was given for mention of MPS type VI. A diagnosis made because of the clinical findings in the absence of any analytical results did not receive any points. The interpretative proficiency for this sample was 65%.

Recommendations*:* confirmation of the diagnosis by enzyme assay (N-acetylgalactosamine-4-sulphatase/arylsulfatase B), mutation analysis (*ARSB* gene), GAG analysis (enzymes) and GAG differentiation were considered helpful.

Overall impression: although overall performance with this straightforward sample was less than ideal with 70%, 20 laboratories satisfactory detected an MPS disorder even though only about half differentiated the subtype.

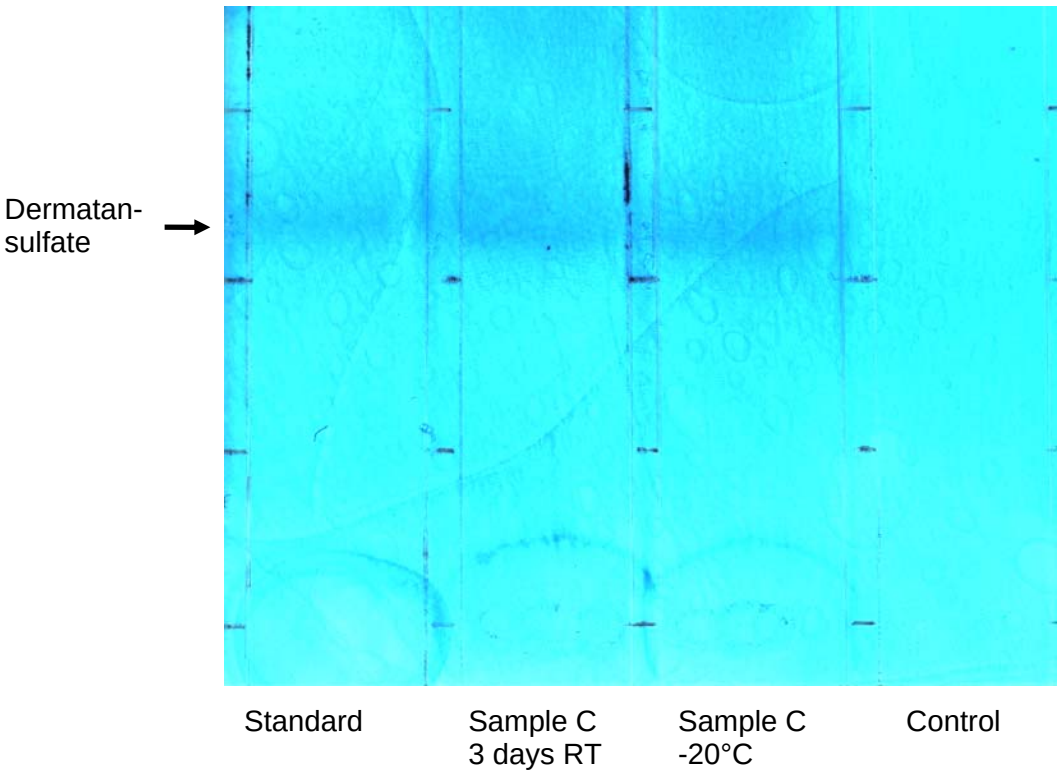
Quantitative data:
Creatinine: mean 1.8, median 1.8, range 1.4 – 2.6

Sample C: GAG and creatinine values



Lab number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
GAG (g/mol creat)	21		19		14	44		27		18	14	24	12	20	19	12	25		17	22		23	13
Creatinine (mmol/l)	1.8		1.7	2.0	1.6	1.8	1.7	1.5	1.6	1.9	1.5	1.8	1.7	1.9	1.9	1.7	1.4	1.9		1.6	1.8		2.6

MPS TLC of sample C



Sample D: Homocystinuria

Patient: the sample was obtained from a 7 year old boy with homocystinuria who was receiving treatment. The diagnosis was based on urine and plasma amino acid analysis and confirmed by enzyme assay. This sample was contributed by Dr. M. Baumgartner, Zurich, Switzerland.

Analytical performance: 23 laboratories reported amino acid analyses, but only 20 were able to correctly identify increases of homocyst(e)ine and methionine which scored 1 point each. The analytical performance of this sample was 83%.

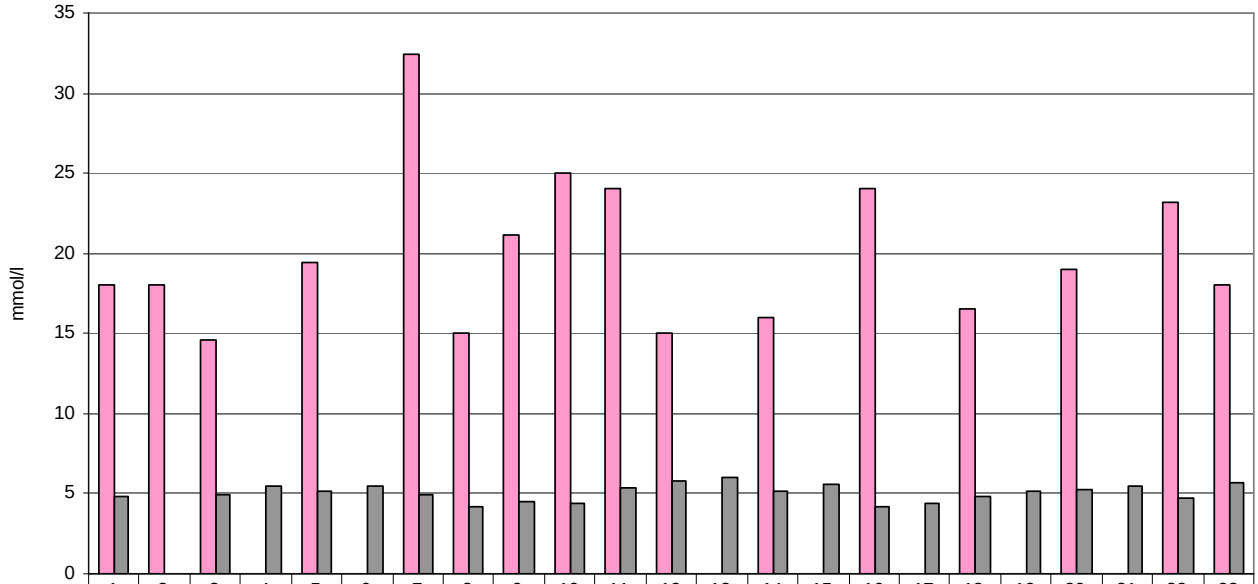
Interpretative proficiency: diagnosis of homocystinuria was considered correct. The proficiency score was 85%.

Recommendations: we consider follow-up by plasma amino acids, enzyme assay (cystathione-ß-synthase) and mutation analysis as important.

Overall impression: fairly good overall performance but although the abnormal levels were not dramatically high it is still disappointing that 3 labs missed the diagnosis.

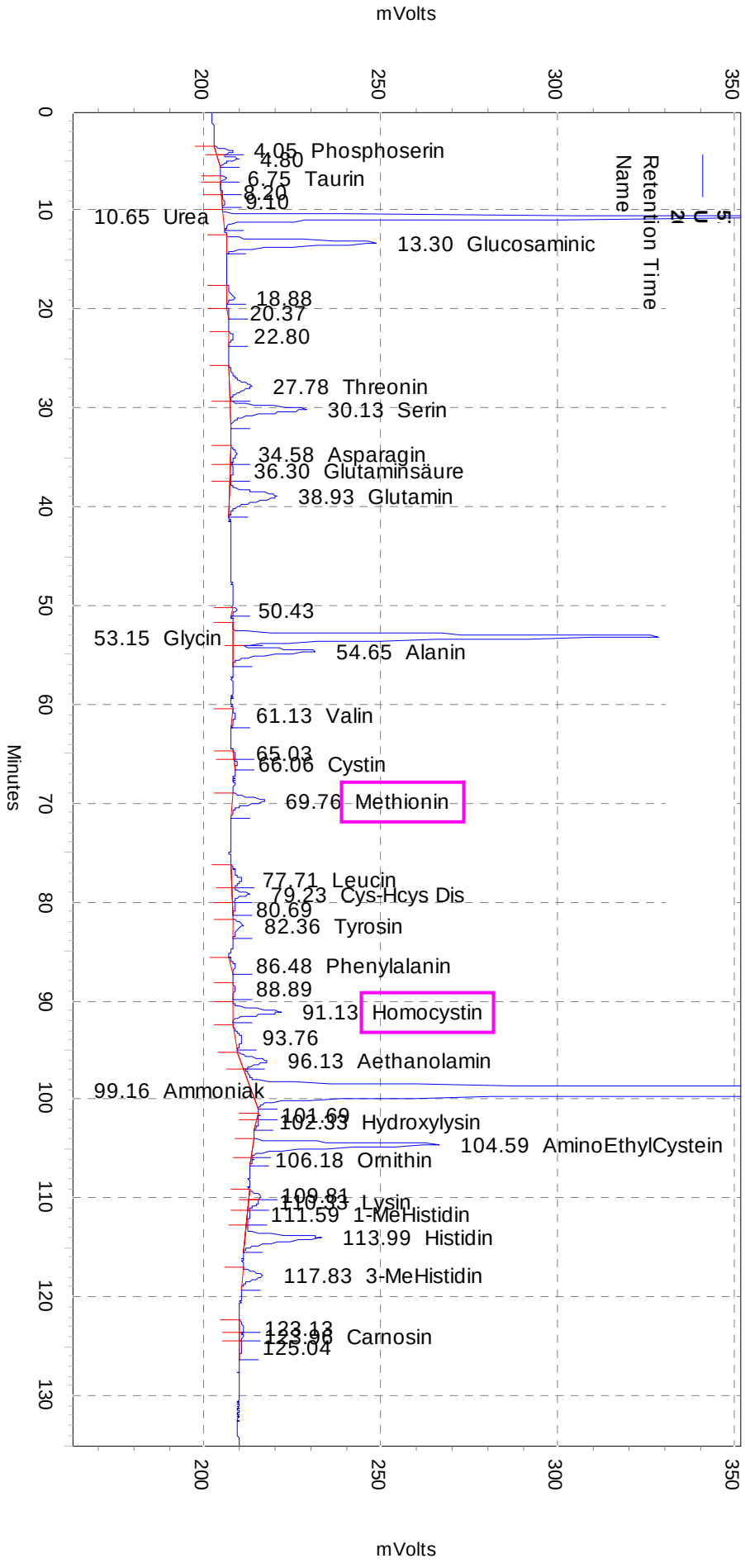
Quantitative data:
Creatinine: mean 5.1, median 5.1, range 4.2 – 6.1

Sample D: Methionine and Creatinine values



Lab number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Methionine	18	18	15		19		32	15	21	25	24	15		16		24		17		19		23	18
Creatinine	4.9		4.9	5.5	5.1	5.5	4.9	4.2	4.5	4.4	5.4	5.8	6.1	5.1	5.6	4.2	4.4	4.8	5.2	5.3	5.5	4.7	5.7

Amino acid chromatogram of sample D (German spelling)



Sample E: Normal

Patient: this sample came from a 13 year old presently healthy child of a member of staff from our hospital. The urine was collected after a chicken meal.

Analytical performance: amino acid and organic acid analysis was considered essential for the diagnosis in this case and was performed by all 23 laboratories. All labs reported either no abnormality or increased excretion of 1-Methyl-Histidine and this was considered correct and received 1 point. All labs reported normal pattern of organic acids and received 1 point. Analytical performance was 100%.

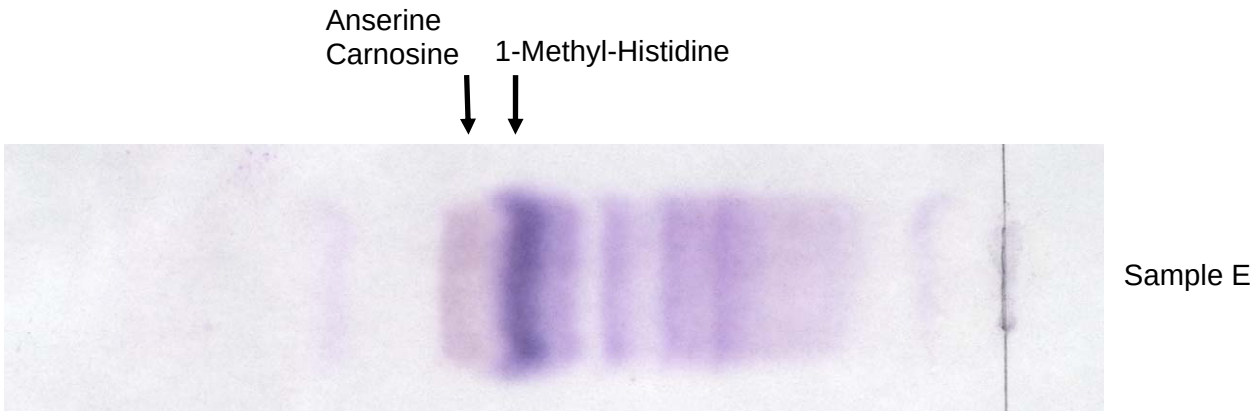
Interpretative proficiency: the diagnosis of no metabolic disorder and connection to poultry intake is considered correct and received 2 points. Proficiency score was 91%.

Recommendations: no further investigations were considered correct.

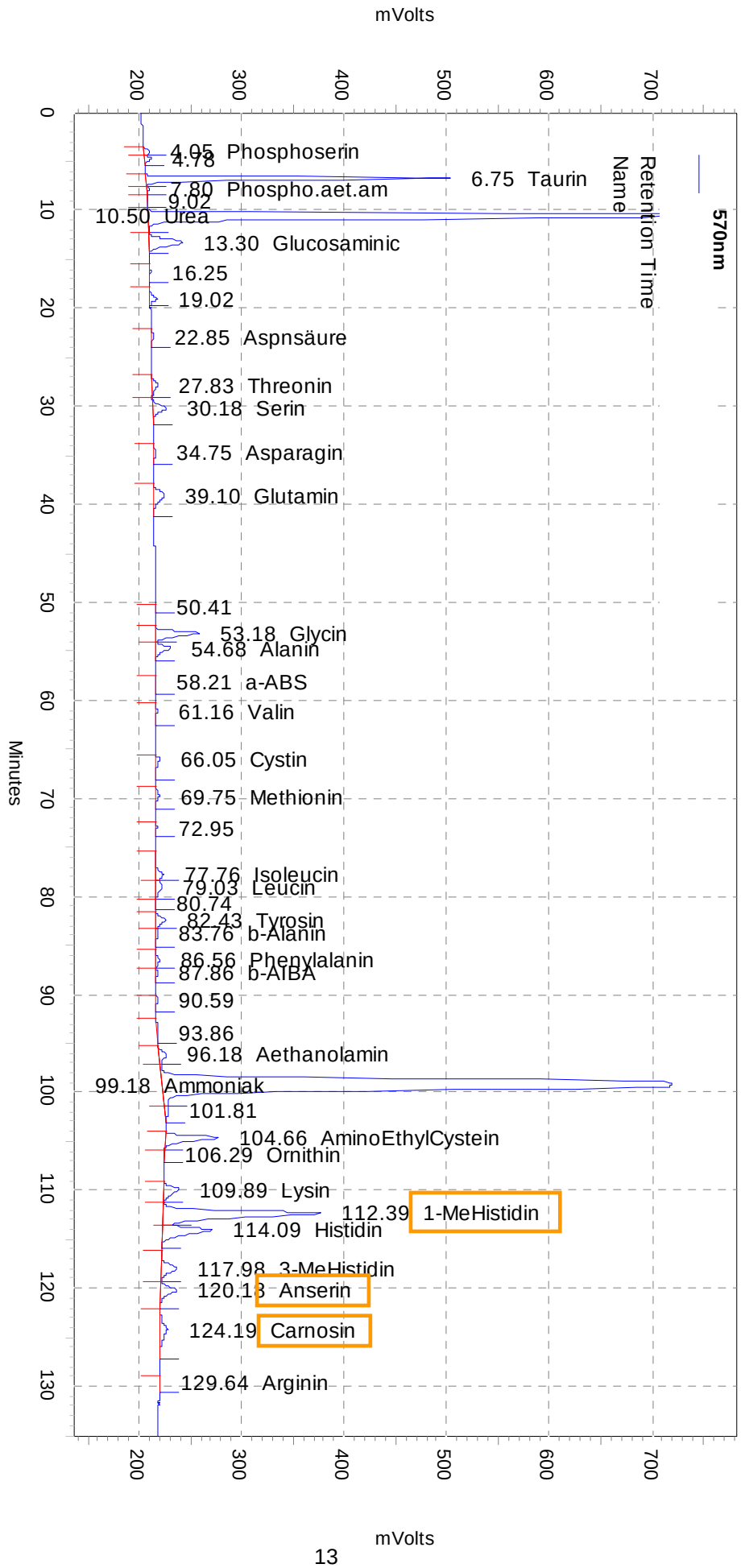
Overall impression: good analytical and interpretative performance, but as usual with “normal” samples a tendency to recommend too many follow-up tests.

Quantitative data:
Creatinine: mean 9.1, median 9.3, range 45.7 – 10.9

High voltage electrophoresis amino acid analysis of sample E



Amino acid chromatogram of sample E (German spelling)



Sample F: Mucopolysaccharidosis type IIIA (Sanfilippo)

Patient: this sample was common to all DPT centres and provided by Dr. V. Kožich, Prague, Czech Republic. It was obtained from a 7 year old boy suffering from mucopolysaccharidosis type IIIA (Sanfilippo). The diagnosis was based on GAG differentiation and had been confirmed enzymatically. The results from all 5 centres were summarised at the ERNIM workshop held in Lisbon, Sept 2, 2008. (See: www.erndim.org / Meetings and Reports.)

Analytical performance*:* mucopolysaccharide analysis was considered essential. The finding of increased total GAG and heparan sulphate following separation was considered correct. 19 laboratories performed mucopolysaccharide analysis. All found increased GAG and/or a positive toluidine blue spot test which received 1 point. An additional point was given for the finding of elevated HS. The analytical performance of this sample was 67%.

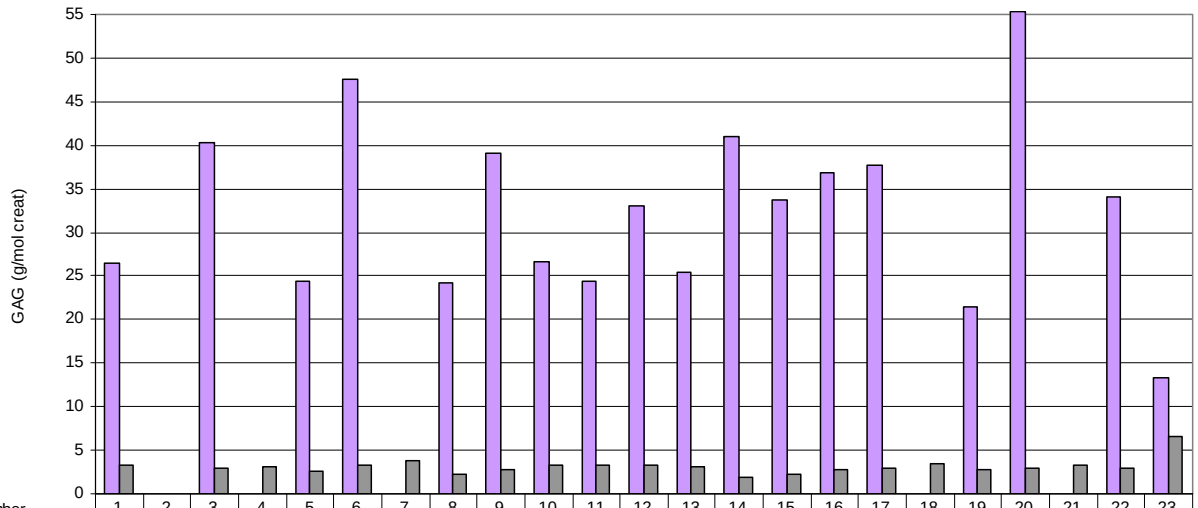
Interpretative proficiency: a diagnosis of MPS in general received 1 point, and 2 points were given for mention of MPS type III (Sanfilippo). A diagnosis made because of the clinical findings in the absence of any analytical results did not receive any points. The interpretative proficiency for this sample was 65%.

Recommendations*:* confirmation of diagnosis by enzyme assay and mutation analysis were considered helpful.

Overall impression: this common sample was of intermediate difficulty. Overall performance was fairly good with only 2 laboratories failing to recognise an MPS disorder although only 11 labs diagnosed the type of MPS.

Quantitative data:
Creatinine: mean 3.1, median 3.1, range 1.9 – 6.5

Sample F: GAG and creatinine values

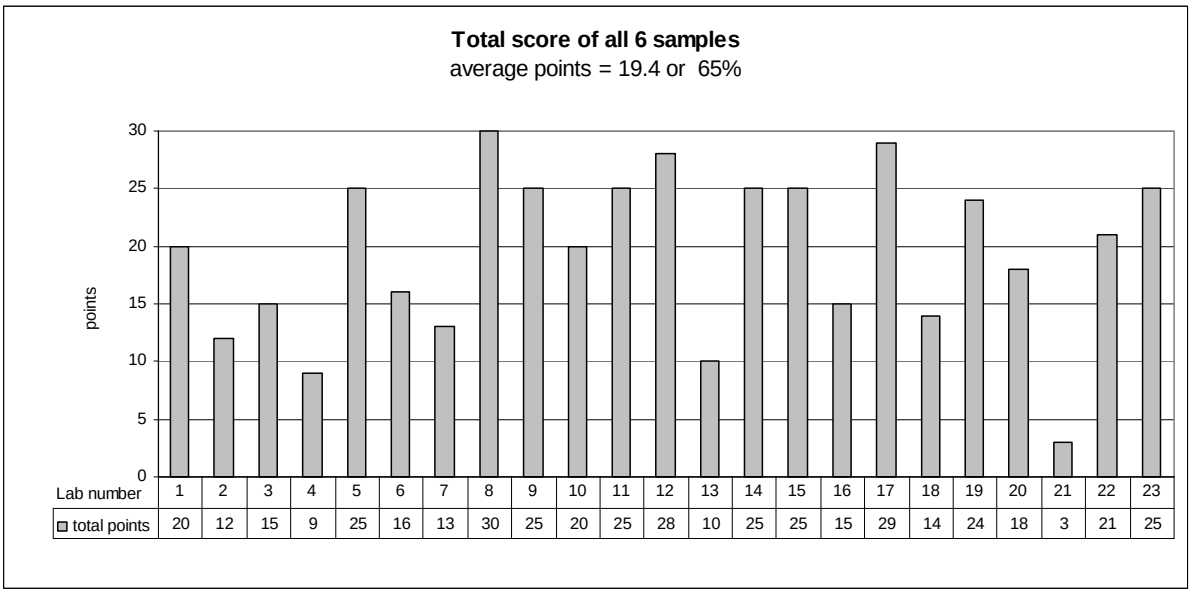


Lab number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
GAG (g/mol creat)	26		40		24	48		24	39	27	24	33	25	41	34	37	38		22	55		34	13
Creatinine (mmol/l)	3.2		3.0	3.1	2.5	3.3	3.8	2.3	2.7	3.3	3.3	3.3	3.1	1.9	2.3	2.8	3.0	3.5	2.8	2.9	3.2	2.9	6.5

9. Scores

Sample	Diagnosis	A (%)	I (%)	R (%)	total (%)
A	Aminoacylase 1 deficiency	35	35	35	**35**
B	ADSL (Adenylosuccinate lyase defic.)	37	37	35	**37**
C	MPS type 6 (Maroteaux-Lamy)	70	65	91	**72**
D	Homocystinuria	83	85	78	**83**
E	Normal	100	91	83	**93**
F	MPS type 3 (Sanfilippo)	67	65	83	**70**

	Survey 1			Survey 2			
Lab no.	**A**	**B**	**C**	**D**	**E**	**F**	**total**
1	0	0	5	5	5	5	**20**
2	0	0	1	5	5	1	**12**
3	0	0	3	5	4	3	**15**
4	0	0	4	0	5	0	**9**
5	5	0	5	5	5	5	**25**
6	0	0	3	5	5	3	**16**
7	0	0	5	3	5	0	**13**
8	5	5	5	5	5	5	**30**
9	5	2	3	5	5	5	**25**
10	0	5	3	4	5	3	**20**
11	5	0	5	5	5	5	**25**
12	5	5	3	5	5	5	**28**
13	0	0	3	0	4	3	**10**
14	0	5	5	5	5	5	**25**
15	5	0	5	5	5	5	**25**
16	0	0	3	5	4	3	**15**
17	5	5	5	4	5	5	**29**
18	0	0	1	5	5	3	**14**
19	5	0	5	4	5	5	**24**
20	0	5	3	5	2	3	**18**
21	0	0	0	0	3	0	**3**
22	0	5	3	5	5	3	**21**
23	0	5	5	5	5	5	**25**



Lab no	Sample A Aminoacylase 1 deficiency				Sample B ADSL				Sample C MPS type 6 (Maroteaux-Lamy)			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	0	0	0	**0**	0	0	0	**0**	2	2	1	**5**
2	0	0	0	**0**	0	0	0	**0**	0	0	1	**1**
3	0	0	0	**0**	0	0	0	**0**	1	1	1	**3**
4	0	0	0	**0**	0	0	0	**0**	2	1	1	**4**
5	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
6	0	0	0	**0**	0	0	0	**0**	1	1	1	**3**
7	0	0	0	**0**	0	0	0	**0**	2	2	1	**5**
8	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
9	2	2	1	**5**	1	1	0	**2**	1	1	1	**3**
10	0	0	0	**0**	2	2	1	**5**	1	1	1	**3**
11	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	1	0	**3**
13	0	0	0	**0**	0	0	0	**0**	1	1	1	**3**
14	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
15	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
16	0	0	0	**0**	0	0	0	**0**	1	1	1	**3**
17	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
18	0	0	0	**0**	0	0	0	**0**	0	0	1	**1**
19	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
20	0	0	0	**0**	2	2	1	**5**	1	1	1	**3**
21	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
22	0	0	0	**0**	2	2	1	**5**	1	1	1	**3**
23	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**
ratio	16/46	16/46	8/23	**40/115**	17/46	17/46	8/23	**42/115**	32/46	30/46	21/23	**83/115**
%	**34.8**	**34.8**	**34.8**	**34.8**	**37.0**	**37.0**	**34.8**	**36.5**	**69.6**	**65.2**	**91.3**	**72.2**

Lab no	Sample D Homocystinuria				Sample E Normal				Sample F MPS type 3 (Sanfilippo)			
	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**	0	0	1	**1**
3	2	2	1	**5**	2	2	0	**4**	1	1	1	**3**
4	0	0	0	**0**	2	2	1	**5**	0	0	0	**0**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
7	2	1	0	**3**	2	2	1	**5**	0	0	0	**0**
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	0	**4**	2	2	1	**5**	1	1	1	**3**
11	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	0	0	0	**0**	2	2	0	**4**	1	1	1	**3**
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	2	1	**5**	2	2	0	**4**	1	1	1	**3**
17	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
18	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
19	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
20	2	2	1	**5**	2	0	0	**2**	2	1	0	**3**
21	0	0	0	**0**	2	0	1	**3**	0	0	0	**0**
22	2	2	1	**5**	2	2	1	**5**	1	1	1	**3**
23	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
ratio	38/46	39/46	18/23	**95/115**	46/46	42/46	19/23	**107/115**	31/46	30/46	19/23	**80/115**
%	**82.6**	**84.8**	**78.3**	**82.6**	**100**	**91.3**	**82.6**	**93.0**	**67.4**	**65.2**	**82.6**	**69.6**

10. Assessment of performance

Steps have been taken within the Scientific Advisory Board of ERNDIM to set the level of good performance within a proficiency scheme. Letters of support to those laboratories with clear poor performance will be issued.

11. Annual meeting

The annual meeting of participants of the DPT centre Basel took place in Lisbon at the SSIEM Annual Symposium on September 2, 2008.

12. Changes planned for 2009

A system for online submission and evaluation of results and reporting is being developed by B. Fowler and V. Kožich and tested on a pilot scale. Participants will be notified of developments in due course.

13. Tentative schedule and fee in 2009

Task	**Due**
Sample distribution	April 20, Monday
Start of analysis of Survey 2009/1	May 04, Monday
Survey 2009/1 - Results submission	May 25, Monday
Survey 2009/1 - Reports	June 15, Monday
Start of analysis of Survey 2009/2	June 22, Monday
Survey 2009/2 – Results submission	July 13, Monday
Survey 2009/2 - Reports	August 07, Friday
Annual meeting of participants	
Annual Report 2009	December

The next annual meeting of DPT participants will be announced.

The Executive Board of ERNDIM determined the fee for 2009 in the amount of 290 €.

14. Certificate of participation

The certificate of participation will be provided by ERNDIM to all participants who returned the results of both surveys. In addition, we are introducing a new type of certificate which will now indicate whether satisfactory performance was achieved in the scheme.

Basel, November 2008

Brian Fowler	Katharina Honegger	Marianne Zaugg
Scientific advisor	Scheme organiser	Scheme organiser