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Diagnostic Proficiency Testing

Survey 2009

Annual Report

prepared by
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1. Geographical distribution of participants

This year, 21 laboratories from 9 countries participated in our scheme. Originally 22 labs registered but 1 lab had subscribed falsely to this scheme.

Country	Number of participants
Austria	1
Canada	3
Estonia	1
Germany	6
Norway	1
Sweden	2
Switzerland	2
UK	1
USA	4

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 20 at ambient temperature using the courier TNT Swiss Post.
Delivery of samples took between 1 and 3 days according to the tracking by the courier, however, the delivery times stated by the participants varied between 1 to 7 days. Unfortunately 3 labs received their samples after more than 7 days due to problems at the US customs.
Nineteen participants returned their results by the deadline, 2 with a short delay. Regardless of the delay all reported results were accepted by the organisers.

4. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/ pyrimidines were required in 2009.

5. Schedule of the scheme 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	October 23, in Basel
Annual Report 2009	December

6. Receipt of samples and results

Date of receipt of samples (sent on April 20, 2009)

Receipt (days after shipment)	Receipt (reported by participants)	Delivery (by TNT Swiss Post)
1 day	7	13
2 days	6	4
3 days	2	1
4 days	1	0
more than 7 days	5	3

Date of reporting of results

Receipt of results	Part 1 (deadline May 25)	Part 2 (deadline July 13)
deadline or before	19 participants	20
1 day delay	-	1
2 days delay	1	-
7 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 or 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: ß-Ketothiolase Deficiency / 3-Oxothiolase Deficiency

Patient: the sample was obtained from a 3 year old girl with ketothiolase deficiency/2-oxothiolase deficiency who was receiving no treatment. The diagnosis was based on urine organic acid analysis, with confirmation by enzyme assay. This sample was contributed by Dr. M. Engval, Huddinge/Stockholm, Sweden.

Analytical performance: 21 laboratories reported organic acid analyses, 20 were able to correctly identify at least 2 of the 3 key metabolites (2-methyl-3-hydroxybutyric acid, Tiglylglycine, 2-methylacetoacetate) which scored 2 points. The analytical performance of this sample was 98%.

Interpretative proficiency: diagnosis of ketothiolase deficiency/2-oxothiolase deficiency was considered correct. The finding of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency was considered to be partially correct and received 1 point. The proficiency score was 95%. In fact, close inspection of the chromatogram reveals two peaks of 2-methyl-3-hydroxy-butyric acid which is indicative of ketothiolase deficiency but not found in the dehydrogenase deficiency (see figure 2).

Recommendations: we consider follow-up by enzyme assay (ß-ketothiolase), mutation analysis (*ACAT1* gene), carnitine and acylcarnitine analysis as important.

Overall impression: relatively straight forward sample with very good analytical and interpretative performance (overall 95%).

Figure 1: Organic acid chromatogram

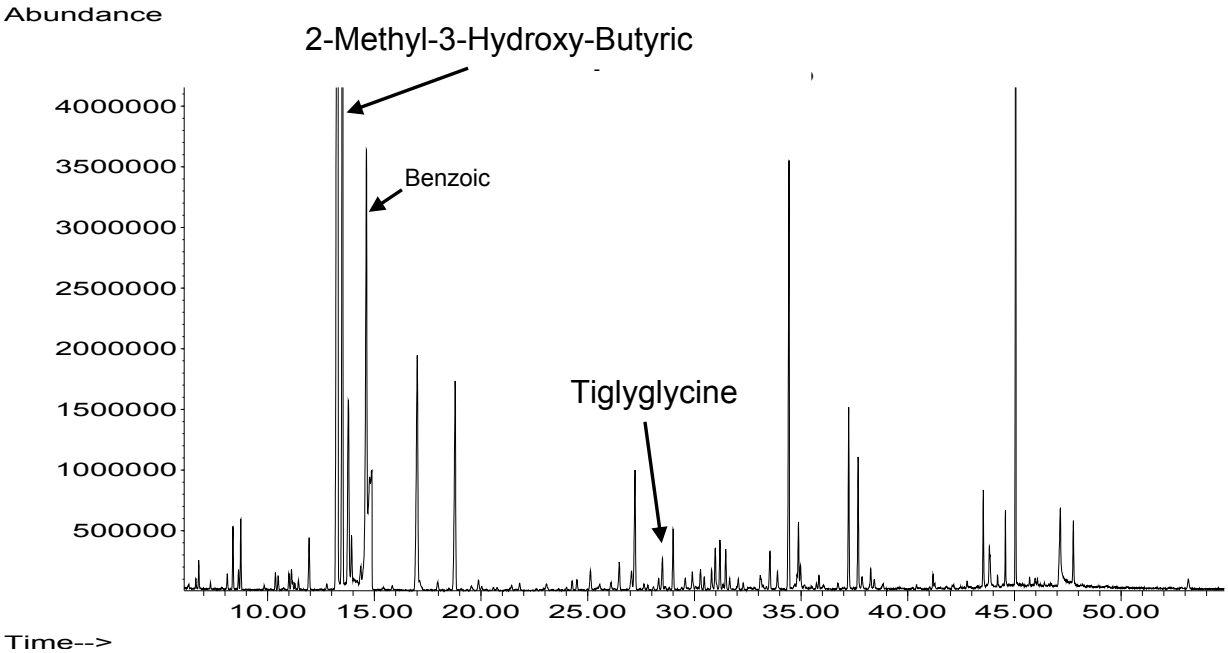
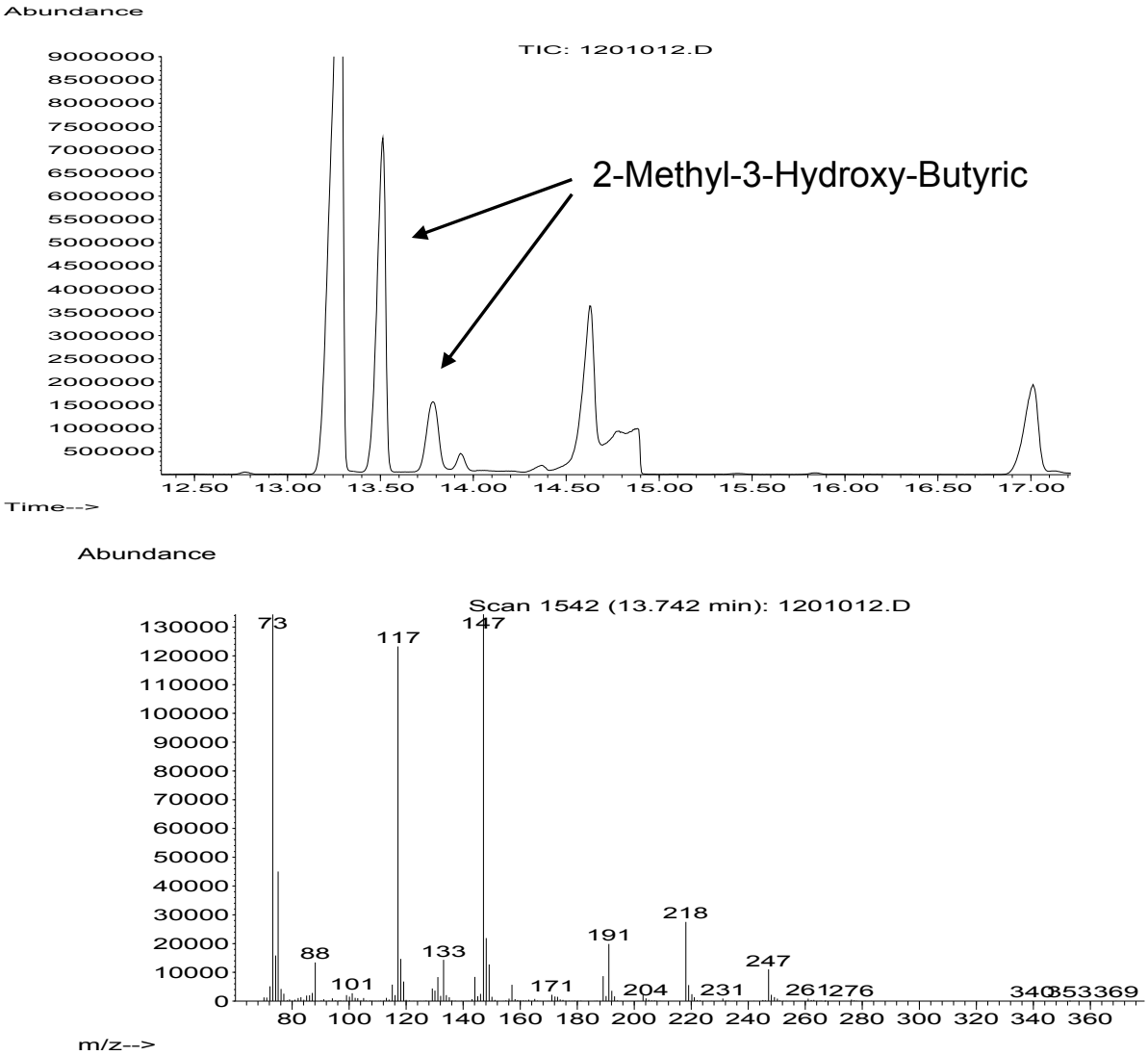


Figure 2: Organic acid chromatogram and spectrum



Sample B: Triple H syndrome (Hyperammonemia-Hyperornithinemia-Homocitrullinuria)

Patient: this sample came from a 26 year old patient with triple H syndrome (Hyperammonemia-Hyperornithinemia-Homocitrullinuria). The patient is under the following treatment: citrulline, creatine, moderate protein restriction. The sample was provided by Dr. C. Dionisi, Rome, Italy. The diagnosis had been confirmed by mutation analysis of the *ORNT1* gene (homozygous c.861insg).

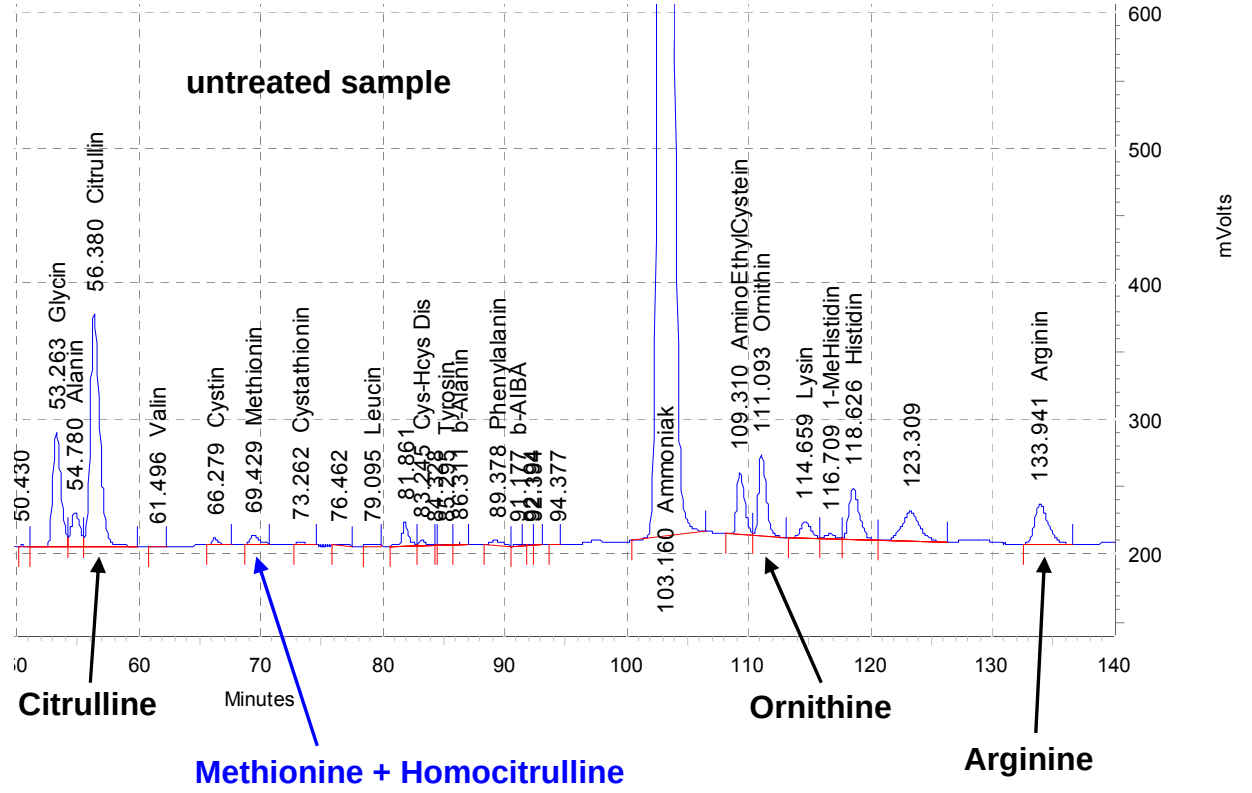
Analytical performance: Amino acid analysis was considered essential for the diagnosis in this case and was performed by 20 laboratories and received 1 point. Although the level of the key metabolite, homocitrulline, was not very high, 3 laboratories correctly identified this. However, since this sample was collected on treatment and therefore not typical we gave 1 point for the finding of abnormal amino acids pointing to a urea cycle defect. The finding of orotic acid and uracil in organic acid and/or purine/pyrimidine analysis also scored 1 point each, as reported by 15 labs (max. points: 2). Analytical performance was 86%.

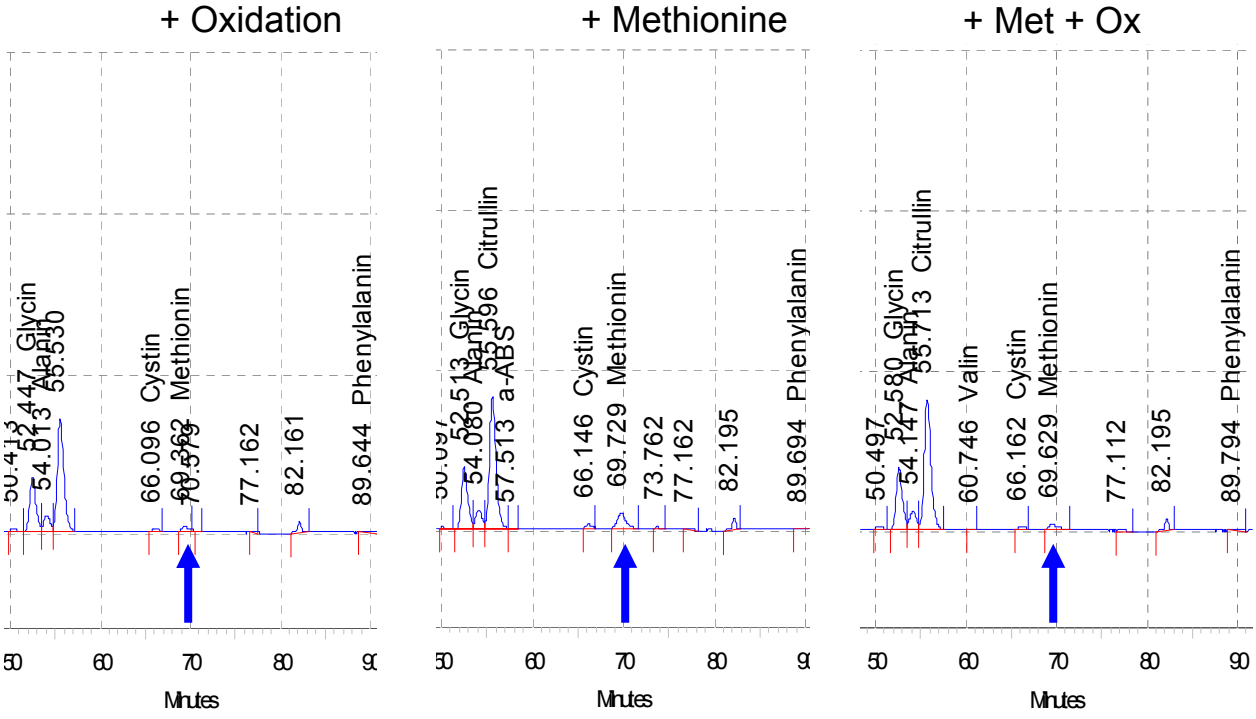
Interpretative proficiency: the diagnosis of triple H syndrome (reported by 4 labs) was considered correct and received 2 points. 1 point was given for indication of another urea cycle disorder. Proficiency score was 57%.

Recommendations: follow-up by plasma amino acid and ammonia analysis was important. Confirmation of diagnosis by enzyme assay (ornithine transporter) and mutation analysis (*ORNT1* gene) were considered helpful.

Overall impression: This was a difficult sample, particularly because the patient was under treatment with citrulline. The analytical performance highlights the difficulty of separating homocitrulline from methionine using conventional ion chromatography. Nevertheless, homocitrulline was identified by 3 labs using either tandem MS or even (in 1 case) ion exchange chromatography. Another possible approach to aid identification could be oxidation using performic acid which destroys methionine but not homocitrulline.

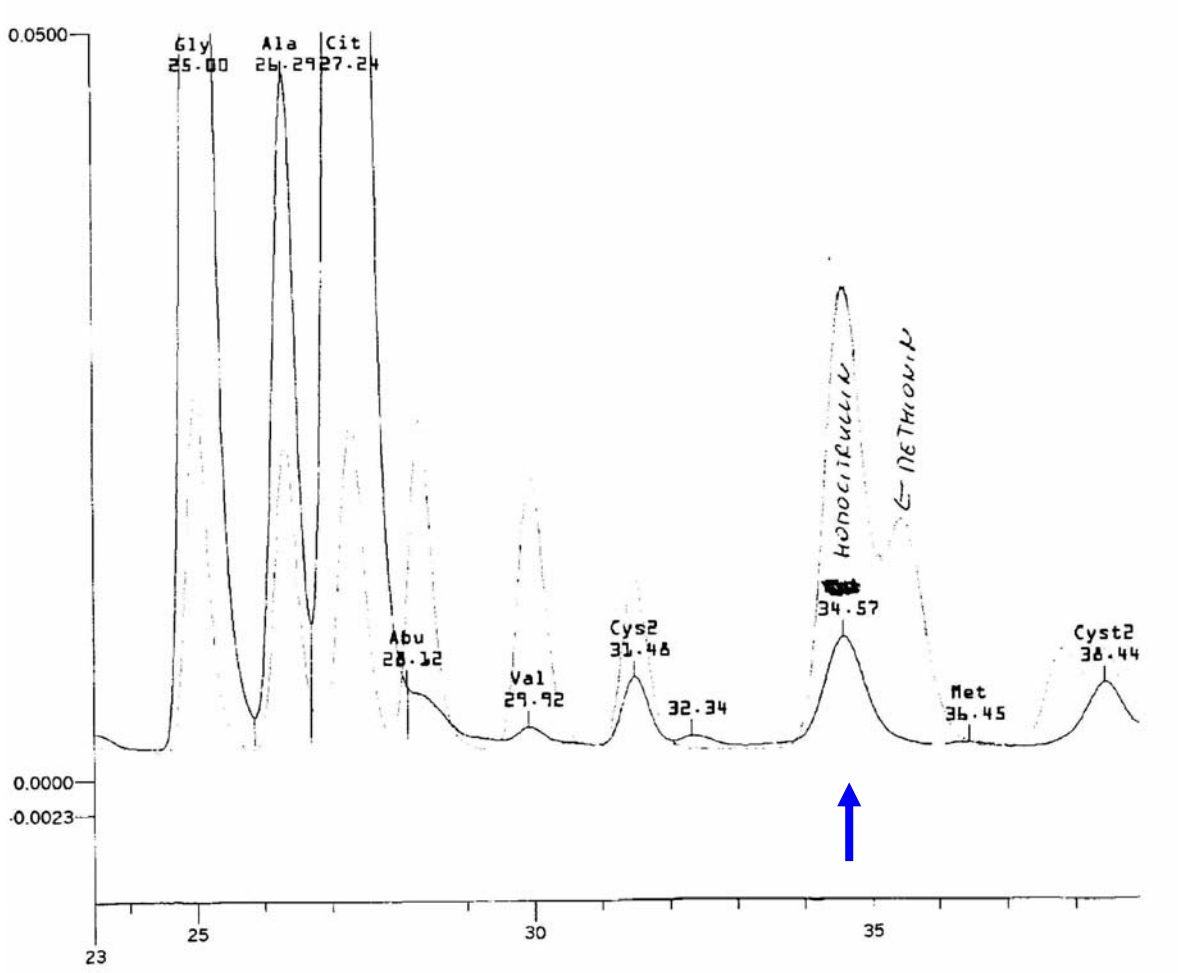
Figure 3: Amino acid chromatogram, +/- oxidation and methionine





Methionine + Homocitrulline peak

Figure 4: Amino acid chromatogram overlaid with homocitrulline standard chromatogram (faint line)



Source Figure 4: participating lab

Sample C: MPS II (Mucopolysaccharidosis type II, Hunter)

Patient: this sample was obtained from a 4.5 year old boy suffering from mucopolysaccharidosis type II (Hunter). This urine was obtained in our hospital, Basel, Switzerland. The diagnosis had been confirmed by the finding of severe iduronate sulphate sulphatase deficiency in serum and leucocytes (Prof. B. Steinmann, Zurich, Switzerland).

Analytical performance*:* mucopolysaccharide analysis was considered essential. The finding of increased GAG, heparan and dermatan sulphate was considered correct. 20 laboratories performed mucopolysaccharide analysis. All found increased GAG which received 1 point, 17 reported quantitative values for GAG (see Figure 5). One additional point was given for GAG differentiation with identification of dermatan sulphate. The analytical performance of this sample was 76%.

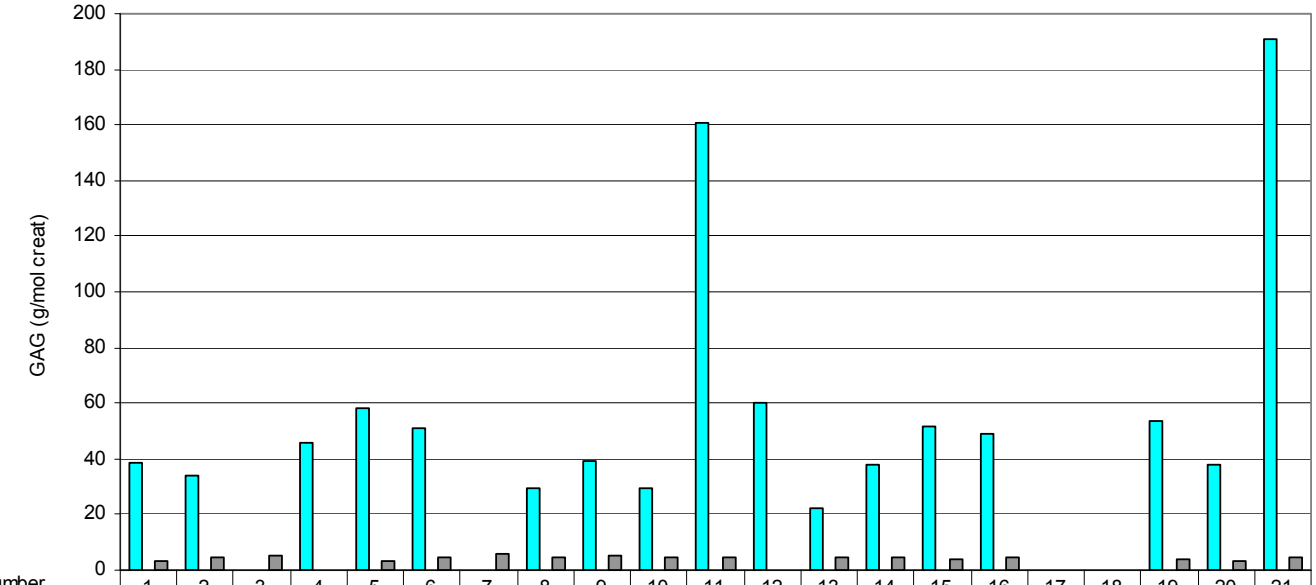
Interpretative proficiency: a diagnosis of MPS in general received 1 point, and 1 point was given for mention of MPS type II. The interpretative proficiency for this sample was 86%.

Recommendations*:* confirmation of diagnosis by enzyme assay (iduronate-2-sulfatase, α-iduronidase), mutation analysis (*ARSB* gene), GAG analysis and differentiation were considered helpful.

Overall impression: The overall performance with this straightforward sample was 84%. All labs which performed MPS analysis satisfactorily detected an MPS disorder and 15 mentioned the diagnosis of MPS II. The variation of GAG values was large, although this was mainly due to 2 particularly high values.

Quantitative data:
mean = 4.6, median = 4.7, range: 3.0 – 5.9

Figure 5: 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
GAG (g/mol creat)	38.65	33.8		46	57.9	50.8		29.65	38.9	29.65	161	60	22	38	51.75	49.1			53.5	38.1	191
Creatinine (mmol/l)	3.42	4.67	5.27		3	4.496	5.91	4.78	5.29	4.62	4.8		4.5	4.32	4.15	4.77		0	4.1	3.372	4.5

Figure 6: MPS TLC

Sample D: Fumarase deficiency

Patient: the sample was obtained from a 20 year old male patient with fumarase deficiency who was receiving no treatment. The diagnosis was based on urine organic acid analysis and confirmation by enzyme assay. This sample was contributed by Dr. Jim. R. Bonham, Sheffield, UK.

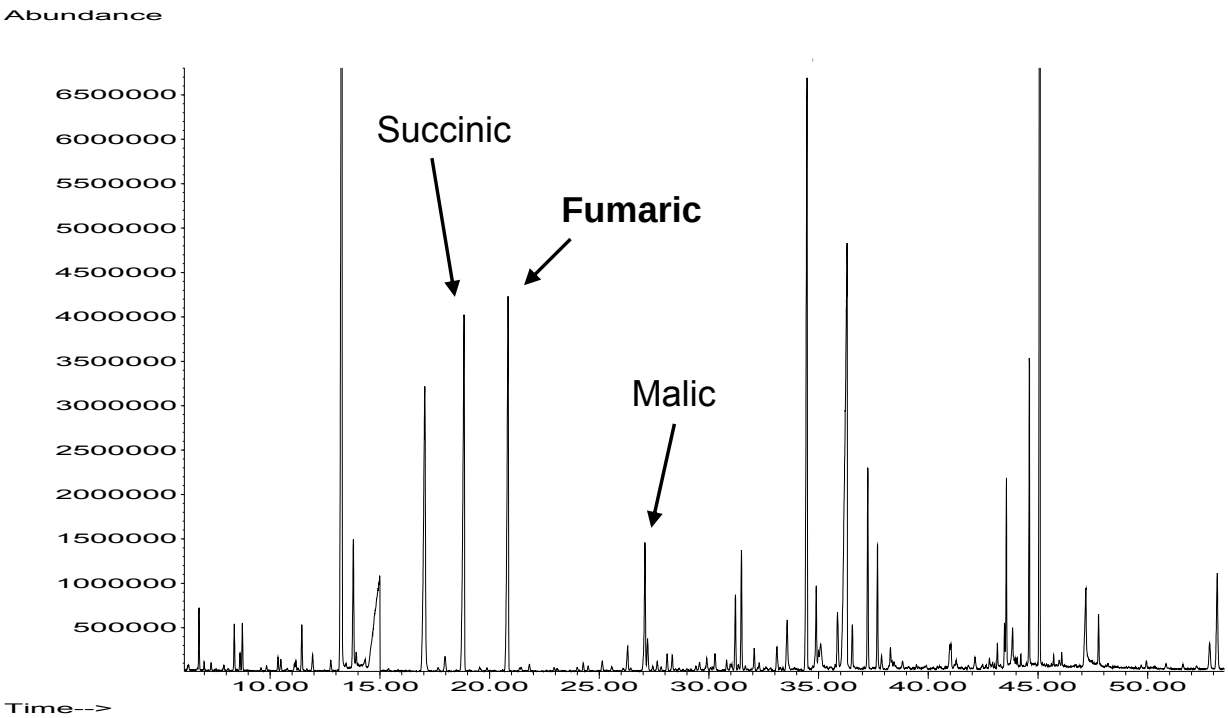
Analytical performance: 21 laboratories reported organic acid analyses, 19 were able to correctly identify the key metabolite fumaric acid with or without other related metabolites (malic acid, succinic acid) which scored 2 points. The analytical performance of this sample was 91%.

Interpretative proficiency: diagnosis of fumarase deficiency was considered correct and received 2 points. A general mitochondrial disorder received 1 point. The proficiency score was 67%.

Recommendations: we consider follow-up by enzyme assay (fumarase/ fumarate hydratase) and mutation analysis (*FH* gene), as important.

Overall impression: fairly good overall performance of 78%. Only 11 labs found the correct diagnosis. Although most labs reliably identified increased amounts of fumaric acid, several were mislead by the presence of malic acid. The presence of malic acid could occur due to the presence of both cytosolic and mitochondrial fumarase activity. Thus, the mitochondrial form may be somewhat deficient. The cumulated fumarate may be converted to malate.

Figure 7: Organic acid chromatogram



Sample E: ASA (Argininosuccinic aciduria, Argininosuccinate lyase deficiency)

Patient: this sample came from a 20 year old male patient with ASA who is under treatment with arginine. This urine was obtained in our hospital, Basel, Switzerland. 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.

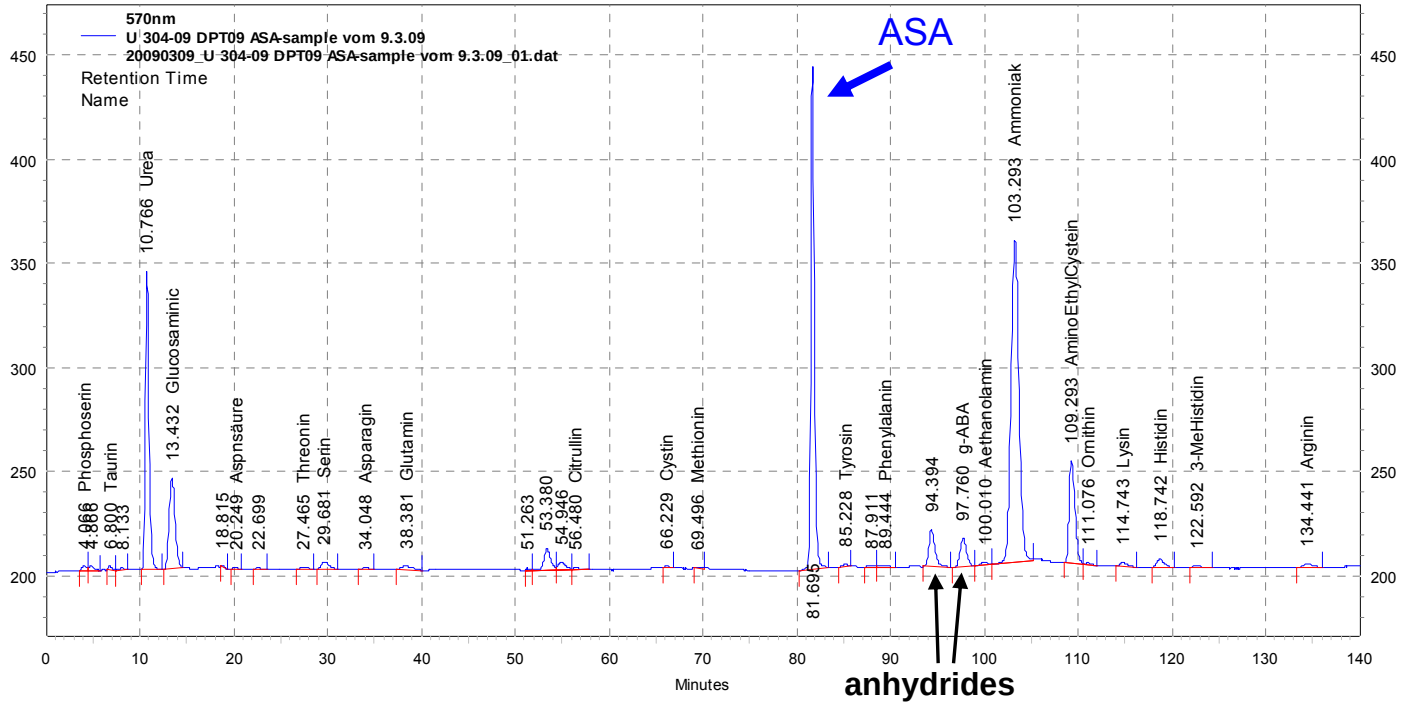
Analytical performance: Amino acid analysis was considered essential for the diagnosis in this case and was performed by 20 laboratories. The finding of argininosuccinate as the key amino acid received 2 points. Anhydrides of argininosucciniate, citrulline and arginine were also considered important. Analytical performance was 95%.

Interpretative proficiency: the diagnosis of ASA was considered correct and received 2 points. Proficiency score was 95%.

Recommendations: follow-up by plasma amino acid and ammonia analysis, confirmation of diagnosis by enzyme assay (argininosuccinate lyase, ASL) and mutation analysis (*ASL* gene) were considered helpful.

Overall impression: 20 out of 21 labs scored the maximum points which led to a very good overall performance of 95%. This sample is the same as that distributed in 2006. At that time proficiency was 75% compared with the improved level of 95% in this round of testing.

Figure 8: Amino acid chromatogram (German spelling)



Sample F: Salla disease, Sialuria

Patient: this was the common sample distributed in all 5 DPT schemes. This sample was obtained from a 6 year old female patient suffering from Salla disease. This urine was provided by Dr. J.-E. Månsson, Molndal, Sweden. The diagnosis had been confirmed by the finding of homozygosity for the Finnish sialin mutation R39C.

Analytical performance*:* the finding of increased free sialic acid using colorimetric, TLC and HPLC methods was considered essential and received 2 points. The analytical performance of this sample was 26%. See figure 9 for a TLC of this sample and aged matched controls.

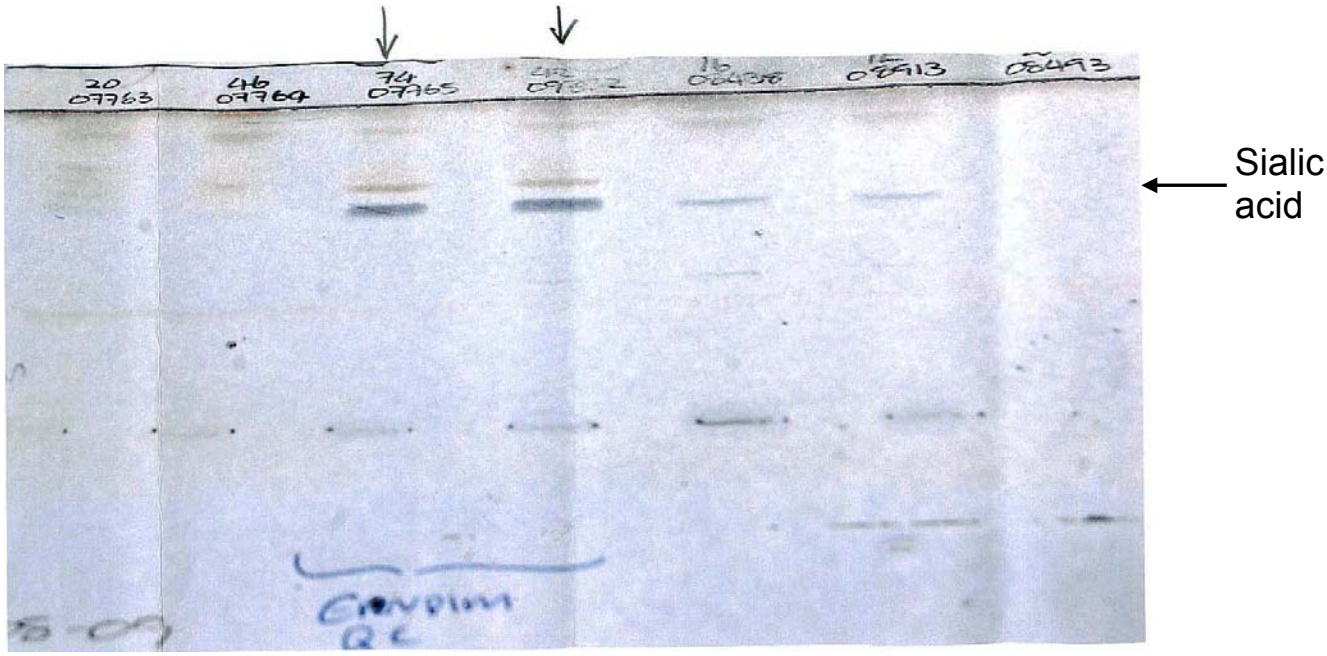
Interpretative proficiency: a diagnosis of Salla received 2 points. The interpretative proficiency for this sample was 24% (5 labs found Salla disease)

Recommendations*:* confirmation of diagnosis by finding of lysosomal membrane protein (sialin) and mutation analysis (SLC17A5 gene) were considered helpful.

This was a difficult sample and required specific testing for sialic acid which could have been selected because of the clinical signs in the patient.

Overall impression: this common sample was of high difficulty. Overall performance was very poor with only 29%. Only 5 labs found the correct diagnosis. Please see the ERNDIM website under Meetings for detailed presentation of this common DPT sample at the ERNDIM meeting held in Basel, October 22-23, 2009 (www.erndim.org).

Figure 9: Sialic acid chromatogram

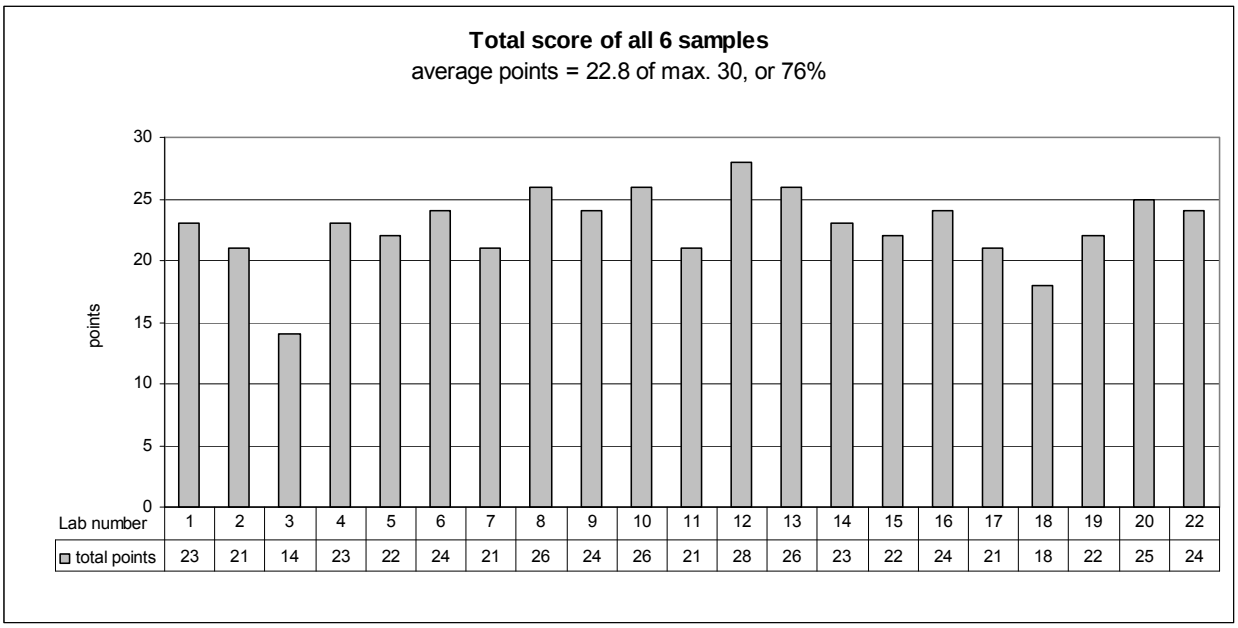


Source Figure 9: Alan Cooper, Manchester, UK

9. Scores

Sample	Diagnosis	A (%)	I (%)	R (%)	total (%)
A	Ketothiolase deficiency	98	95	91	**95**
B	Triple H syndrome	86	57	86	**74**
C	MPS type 2 (Hunter)	76	86	95	**84**
D	Fumarase deficiency	91	67	76	**78**
E	ASA	95	95	95	**95**
F	Salla disease	26	24	43	**29**

	Survey 1			Survey 2			
Lab no.	**A**	**B**	**C**	**D**	**E**	**F**	**total**
1	5	4	5	4	5	0	**23**
2	4	4	4	4	5	0	**21**
3	5	1	3	5	0	0	**14**
4	5	4	4	5	5	0	**23**
5	5	5	2	5	5	0	**22**
6	4	4	4	5	5	2	**24**
7	5	3	3	0	5	5	**21**
8	5	4	5	2	5	5	**26**
9	5	4	5	5	5	0	**24**
10	5	4	5	2	5	5	**26**
11	4	4	3	5	5	0	**21**
12	5	4	4	5	5	5	**28**
13	5	4	4	3	5	5	**26**
14	5	4	5	4	5	0	**23**
15	5	3	5	4	5	0	**22**
16	4	5	5	4	5	1	**24**
17	5	4	2	5	5	0	**21**
18	4	3	5	0	5	1	**18**
19	5	2	5	5	5	0	**22**
20	5	4	5	5	5	1	**25**
22	5	4	5	5	5	0	**24**



Lab no	Sample A Ketothiolase deficiency				Sample B Triple H syndrome				Sample C MPS type 2 (Hunter)			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	2	1	1	4	2	2	1	5
2	2	2	0	4	1	2	1	4	1	2	1	4
3	2	2	1	5	1	0	0	1	1	1	1	3
4	2	2	1	5	2	1	1	4	1	2	1	4
5	2	2	1	5	2	2	1	5	1	1	0	2
6	2	1	1	4	2	1	1	4	1	2	1	4
7	2	2	1	5	1	1	1	3	1	1	1	3
8	2	2	1	5	2	1	1	4	2	2	1	5
9	2	2	1	5	2	1	1	4	2	2	1	5
10	2	2	1	5	2	1	1	4	2	2	1	5
11	2	2	0	4	2	1	1	4	1	1	1	3
12	2	2	1	5	2	1	1	4	1	2	1	4
13	2	2	1	5	2	1	1	4	2	1	1	4
14	2	2	1	5	2	1	1	4	2	2	1	5
15	2	2	1	5	2	1	0	3	2	2	1	5
16	2	1	1	4	2	2	1	5	2	2	1	5
17	2	2	1	5	2	1	1	4	0	1	1	2
18	1	2	1	4	1	1	1	3	2	2	1	5
19	2	2	1	5	1	1	0	2	2	2	1	5
20	2	2	1	5	1	2	1	4	2	2	1	5
22	2	2	1	5	2	1	1	4	2	2	1	5
ratio	41/42	40/42	19/21	100/105	36/42	24/42	18/21	78/105	32/42	36/42	20/21	88/105
%	98	95	91	95	86	57	86	74	76	86	95	84

Lab no	Sample D Fumarase deficiency				Sample E ASA				Sample F Salla disease			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	1	1	4	2	2	1	5	0	0	0	0
2	2	1	1	4	2	2	1	5	0	0	0	0
3	2	2	1	5	0	0	0	0	0	0	0	0
4	2	2	1	5	2	2	1	5	0	0	0	0
5	2	2	1	5	2	2	1	5	0	0	0	0
6	2	2	1	5	2	2	1	5	1	0	1	2
7	0	0	0	0	2	2	1	5	2	2	1	5
8	2	0	0	2	2	2	1	5	2	2	1	5
9	2	2	1	5	2	2	1	5	0	0	0	0
10	2	0	0	2	2	2	1	5	2	2	1	5
11	2	2	1	5	2	2	1	5	0	0	0	0
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	1	1	4	2	2	1	5	0	0	0	0
15	2	1	1	4	2	2	1	5	0	0	0	0
16	2	1	1	4	2	2	1	5	0	0	1	1
17	2	2	1	5	2	2	1	5	0	0	0	0
18	0	0	0	0	2	2	1	5	0	0	1	1
19	2	2	1	5	2	2	1	5	0	0	0	0
20	2	2	1	5	2	2	1	5	0	0	1	1
22	2	2	1	5	2	2	1	5	0	0	0	0
ratio	38/42	28/42	16/21	82/105	40/42	40/42	20/21	100/105	11/42	10/42	9/21	30/105
%	91	67	76	78	95	95	95	95	26	24	43	29

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. This year 18 points or more has been set as the level for satisfactory performance. Thus, only 1 lab did not reach this level.

11. Annual meeting

The annual meeting of participants of the 5 DPT centres took place in Basel Switzerland at the ERNDIM meeting on October 23, 2009. Twelve participants and 2 guests attended the meeting of the Basel centre. Agreement was received for the allocated points.

12. Changes planned for 2010

We plan important changes for 2010.
First you will be requested to submit results online to our website. You will be contacted soon with more details and you will be given the opportunity to practice using the website with 2009 data.
Second as part of the professionalisation of our schemes it is the intention of the ERNDIM board to delegate the administrative aspects of the DPT schemes to an accredited organisation i.e. the Swiss Centre for Quality Control (CSCQ) in Geneva in a similar role to that of SKML for the quantitative schemes. As the first step we will collaborate with CSCQ just for the Basel scheme. This means that you will receive samples from CSCQ but we will remain responsible for the scientific and evaluation aspects of the scheme.

13. Tentative schedule and fee in 2010

Task	**Due**
Sample distribution	May 3, Monday
Start of analysis of Survey 2010/1	May 17, Monday
Survey 2010/1 - Results submission	June 7, Monday
Survey 2010/1 - Reports	June 28, Monday
Start of analysis of Survey 2010/2	June 28, Monday
Survey 2010/2 – Results submission	July 19, Monday
Survey 2010/2 - Reports	August 13, Friday
Annual meeting of participants	August 31, Istanbul
Annual Report 2010	December

The next annual meeting of DPT participants will take place on August 31 in Istanbul Turkey at the SSIEM meeting.

The Executive Board of ERNDIM determined the fee for 2010 in the amount of 320 €.

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.

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