ERNDIM DPT Centre

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Diagnostic Proficiency Testing

Survey 2013

Final Report

prepared by
Brian Fowler

1. Geographical distribution of participants

In 2013, 19 laboratories from 10 countries subscribed to the scheme. For both surveys 19 laboratories submitted results.

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

2. Samples and Shipment

The samples contain a small amount of thiomersal 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. The urine samples were distributed to participants on April 29th at ambient temperature by CSCQ using the courier DHL. Delivery times of samples reported by the courier ranged from 1 to 5 days. There were discrepancies with times reported by participants suggesting possible internal delays.

3. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/ pyrimidines were required in 2013.

4. Schedule of the scheme in 2013

Sample distribution	April 29, 2013
Start of analysis of Survey 2013/1	May 13, 2013
Survey 2013/1 - Results submission	June 3, 2013
Survey 2013/1 - Reports	June 24, 2013
Start of analysis of Survey 2013/2	July 1, 2013
Survey 2013/2 – Results submission	July 22, 2013
Survey 2013/2 - Reports	August 16, 2013
Annual meeting of participants	ICIEM, Barcelona, September 3, 2013
Annual Report 2013	December 2013.

This year we were able to use the evaluation programme to generate individual lab reports and these were distributed in good time on June 28th and August 19. Feedback on the content and style of these reports is invited.

5. Receipt of samples and results

Receipt of samples (sent on April 29, 2013)

Receipt (days after shipment)	No. Labs	Delivery reported by DHL
1	8	11
2	2	6
3	5	2
4	3	
?	1	

Date of reporting of results

19 of 19 labs returned results for both surveys, mainly by the deadline.

6. Scoring system

Two criteria are evaluated: analytical performance, interpretative proficiency including 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 & Recommendations	Good (diagnosis was established)	2	max 2
		Helpful but incomplete	1	
		Misleading or wrong diagnosis	0	

The **total score** is calculated as a sum of these two criteria. The maximum to be achieved is 4 points per sample. The scores were calculated only for laboratories submitting results.

7. Results of samples and evaluation of reporting

Sample A: Isolated methylmalonic aciduria due to the cblA defect (OMIM 251100)

Patient details provided
A female with delayed mental development, episodes of lethargy and vomiting. Currently has secondary nephropathy, chronic interstitial nephritis with reduced glomerular filtration. Has been on weekly dialysis since 2000, shows arterial hypertension. Urine collected at 36 years of age whilst on treatment

This female patient has isolated methylmalonic aciduria due to the cblA defect with a partial response to vitamin B12. First presentation at 3 days and diagnosed at 8 months of age. Despite treatment developed progressive renal insufficiency from the age of 12 years. From the age of 29 on weekly dialysis. Underwent kidney transplantation at 37 years of age with good progress to the present age of 43.
Diagnosis confirmed by fibroblast studies and mutation analysis (homozygous for c.433C>T of the MMAA gene, accession number, 607481).

Analytical performance: Two points were given for identification of MMA. All laboratories correctly identified a moderate increase of methylmalonic acid which scored two points.

Interpretative proficiency: Correct interpretation was considered to be a diagnosis of isolated methylmalonic aciduria with recommendations for further differentiation of the defect AND either reporting of no increase of homocyst(e)ine in this urine or recommendation for plasma homocysteine measurement. Thus 13 labs scored 2 points and 6 one point.

Overall impression:
Good analytical and interpretative proficiency of this straightforward sample although due to treatment, excretion levels relatively low. Nevertheless overall proficiency was good at 92%.

Analytical Details

Creatinine
n=18 (one value 0.5)
median= 3.40
mean= 3.42
SD= 0.03
min, max= [3.05, 3.67]

pH
n=10
median= 6.00
mean= 6.10
SD= 0.04
min, max= [6.00, 6.50]

Spot tests
All negative

Aminoacid analysis

Glycine
n=9
median= 291.90
mean= 312.52
SD= 47.77
min, max= [270.80, 432.00]

Homocystine
n=1
median= 0.00
mean= 0.00
SD= 0.00
min, max= [0.00, 0.00]

Homocysteine	
n=4	
not detected	2
normal, 2.25	1
elevated, 0.73	1

Organic acid analysis (n= 19)

	n	points
MMA moderately increased	19	2

Organic acids column chromatography/**methylmalonic acid**
n=13
median= 146.00
mean= 158.38
SD= 45.05
min, max= [111.00, 280.00]

Organic acids column chromatography/**methyl citric acid**
n=6
median= 27.50
mean= 27.83
SD= 4.59
min, max= [20.00, 35.00]

Interpretation

	n	Points
MMA due to various cause mentioning or excluding combined MMA/HCY	13	2
MMA due to various cause mentioning or excluding combined MMA/HCY	5	1
CblC defect (hcy said to be increased)	1	1

Sample B: citrullinaemia due to argininosuccinate synthetase (EC No.6.3.4.5) deficiency. OMIM No.215700.

Patient details provided:
A male, first seen in the first week of life with increasing sleepiness, mild jaundice and increased liver function tests. Collected at 27 y on treatment

This sample was obtained from a 27 year old male, diagnosed in the first week of life with citrullinaemia, treated with benzoate. The diagnosis was confirmed by mutation analysis. The urine was obtained in the Children's Hospital, Basel, Switzerland. The sample was also distributed in 2010.

Analytical performance*:* Amino acid analysis was considered essential for the diagnosis in this case and was performed by 19 laboratories, all identifying greatly increased citrulline receiving 2 points. Orotic acid was reported as normal (6 labs), increased (6 labs) and increased hippuric acid and benzoic acid were noted reflecting treatment but not considered essential for the diagnosis. Analytical performance was high at 100%.

Interpretative proficiency:
All labs except one gave citrullinaaemia as a likely diagnosis (2 points), although one gave citrin deficiency as most likely. One identified the case as triple H syndrome (no points).

Overall impression
This is a straightforward sample, analytical proficiency 100% but interpretation 95%.

Analytical Details

Creatinine
n=18 (1 value 0.5)
median= 3.89
mean= 3.83
SD= 0.12
min, max= [3.16, 4.33]

pH
n=10
median= 7.00
mean= 6.90
SD= 0.09
min, max= [6.00, 7.00]

Spot tests
All negative

Amino acid analysis (n=19)

	n	points
Citrulline increased	19	2

Amino acid quantitative**/ Citrulline**
n=19
median= 4268.00
mean= 4005.82
SD= 1879.88
min, max= [**1.70**, 6755.00]

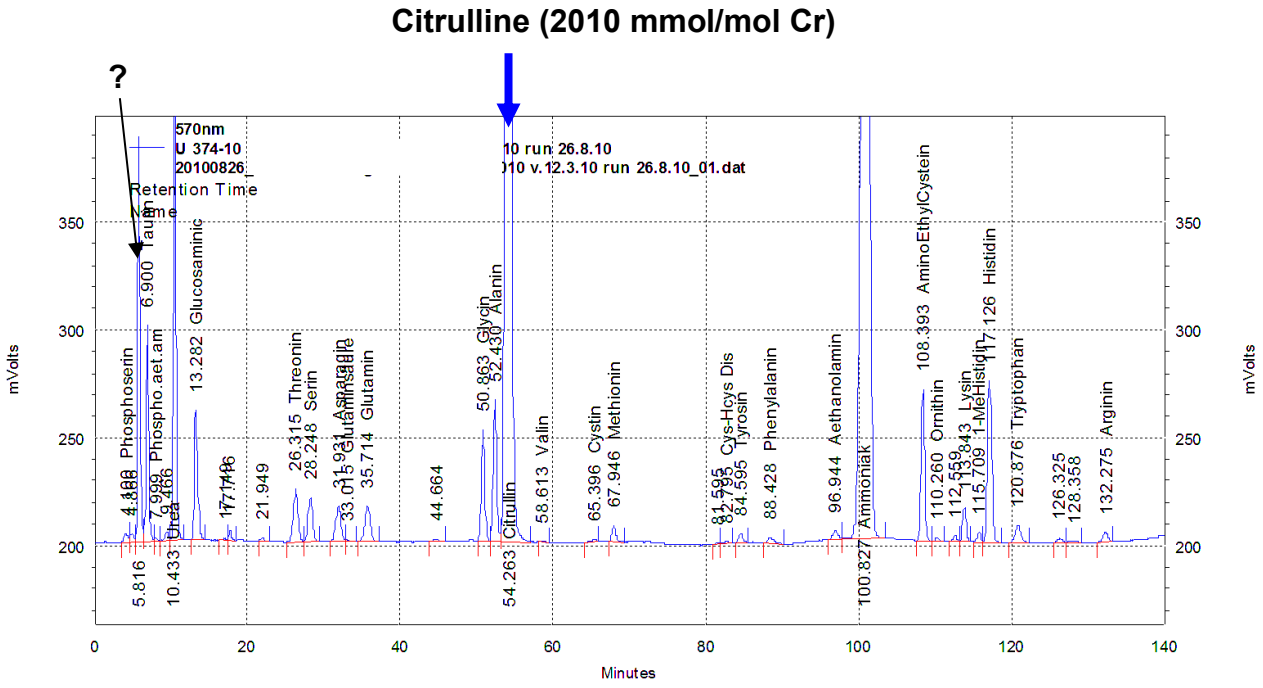
Amino acid quantitative / **Arginine**
n=9
median= 16.00
mean= 14.98
SD= 4.84
min, max= [4.79, 22.00]

Orotic acid
n=7
median= 2.80
mean= 3.10
SD= 3.52
min, max= [0.00, 6.10]

Organic acids column chromatography/**Orotic acid**
n=3
median= 3.00
mean= 2.63
SD= 2.01
min, max= [0.00, 4.90]

	n	Points
Citrullinaemia	18	2
HHH syndrome	1	0

The ion exchange amino acid chromatogram (Biochrom) shows a large peak which elutes immediately before taurine. A paper describes a cylic derivative of citrulline which may well be this substance. (Wilson RW, Gates SC, Kohrman AF, Biochem Med. 1978; 19:90-4).



Sample C: GM1 gangliosidosis (OMIM 230500) due to B-galactosidase (EC 3.2.1.23) deficiency

Patient details provided:
A male patient who came to attention at the age of one month due to oedema associated with hepato-splenomegaly. Urine collected at10 months of age.

A male patient who came to attention at the age of one month due to oedema associated with hepato splenomegaly. Blood film showed unspecific storage vacuoles in lymphocytes. Subsequently showed signs of neuro-degeneration and skin abnormalities. Beta-Galactosidase deficiency found in leucocytes. Receiving symptomatic treatment.

Analytical performance*:* 12 labs that performed oligosaccharide analysis correctly identified an abnormal pattern, 9 as GM1 gangliosidosis (2 points) and 3 with pattern not specified or the wrong type (1 point). This analysis failed in one lab and was not performed or is unavailable in the other 6 labs (0 point). One lab reported elevated homocysteine and another elevated GAGs.

Interpretative proficiency: 12 labs correctly diagnosed GM1 gangliosidosis (2 points)

Overall impression
This sample illustrates the need to either perform oligosaccharide analysis themselves or to link in with a cluster laboratory.

Analytical Details

Creatinine
n=18 (one value 0.1)
median= 0.70
mean= 0.68
SD= 0
min, max= [0.57, 0.75]

pH
n=11
median= 7.00
mean= 7.09
SD= 0.29
min, max= [6.00, 8.00]

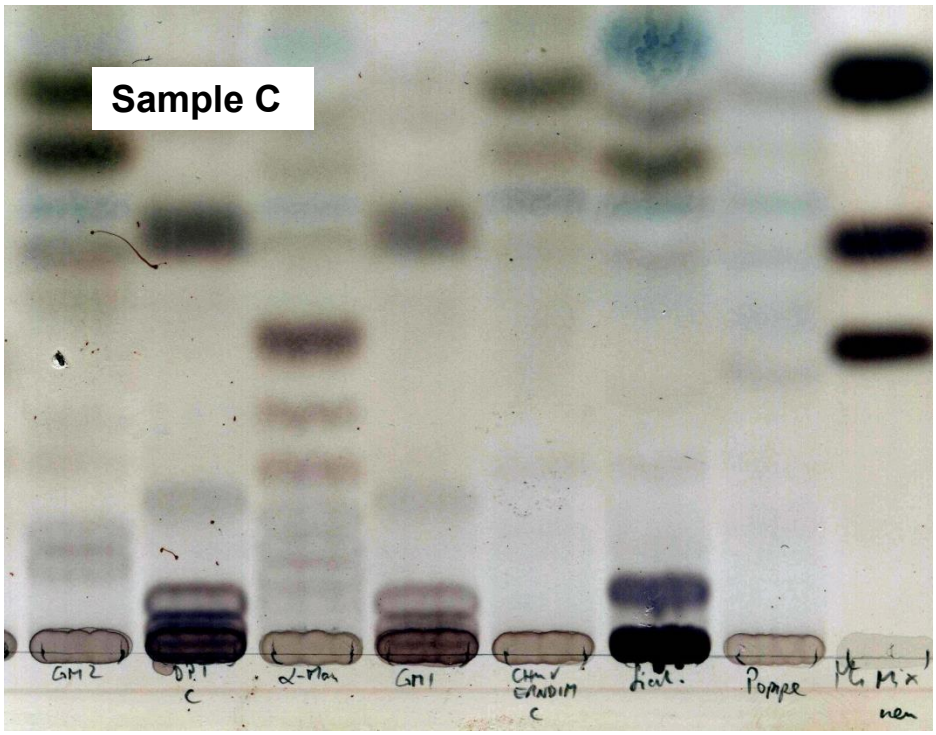
Spot tests
All negative

Oligosaccharides	
Normal profile:	1
Borderline	0
Abnormal profile GM1 specified (2 points)	9
Abnormal profile not specified or wrong type (1 point)	3
Total	13

Interpretation

	n	Points
GM1 gangliosidosis	12	2
No disorder	7	0

Sample C- oligosaccharide analysis



Participant Correspondence on Oligosaccharide analysis

"We are not performing anymore oligosaccharides analysis in our clinical practice, due to the high number of false positives and false negatives with the TLC method.
We have the project to do it by LC/MS eventually. The other problem was that clinicians used to ask for specific enzymes when the clinical symptoms were relatively obvious, but were asking for oligosaccharides when the clinical symptoms were less suggestive, likely giving an even higher number or false negative in these cases. For the moment, as we do most enzymes assays in house, we ask the clinician to order all relevant enzymes, which works well. As we can usually go down to 3mL of blood to isolate leucocytes, this is generally not a problem even in newborns.

We may be too pessimistic regarding the oligosaccharides, but as 6 of the 18 labs did not reported oligosaccharides results for sample C, it may be interesting to ask them why they did not submit results and if they would normally have referred to an external lab. It may be also interesting to look at the Basel results from all participants and see how many reported abnormal results or unknown bands of possible clinical significance in patients without LSDs.
This being said, the Basel program is great and we don't mind having 1-2 samples for which we cannot adequately report results. "

Sample D: 3-Methylglutaconic aciduria in association with Barth Syndrome (OMIM 302060).

Patient detail provided:
8 year old male who presented soon after birth with cardiomyopathy and slightly increased blood lactate. Underwent heart transplantation at 8 months of age. Urine collected at 8y under treatment.

This patient presented at 3 days of life with persistent acidosis with high lactate, cardiomyopathy then heart transplantation. A suspected mitochondrial disorder had led to cardiolipin analysis in cultured fibroblasts (M. Duran, Academic Medical Center Amsterdam showing strongly decreased level of tetralinoleylcardiolipin which was highly suggestive of the diagnosis of Barth syndrome. This was confirmed by the finding of a mutation, c.280C>T (p.Arg94Cys) in the TAZ gene (taffazin) on chromosome Xq28, which has been described before (Lekanne and Mannens, Academic Medical Center Amsterdam).

Analytical performance: The finding of increased 3-methylglutaconic acids was the key abnormality (found by 13 / 19 labs).

Interpretative proficiency:
The correct diagnosis 3-Methylglutaconic aciduria with or without specifying Barth Syndrome was considered to be correct and was made by 14/19 labs.

Overall impression: This was a fairly difficult sample requiring good quantitation of 3-methylglutaconic acid with overall proficiency of 70%

Analytical Details

Creatinine
n=18 (one value 0.022)
median= 2.34
mean= 2.32
SD= 0.08
min, max= [1.30, 2.60]

pH
n=10
median= 6.50
mean= 6.45
SD= 0.19
min, max= [6.00, 7.00]

Spot tests
Sulphite
0: 3
Trace: 1

All others negative

Organic acid analysis (n= 20)

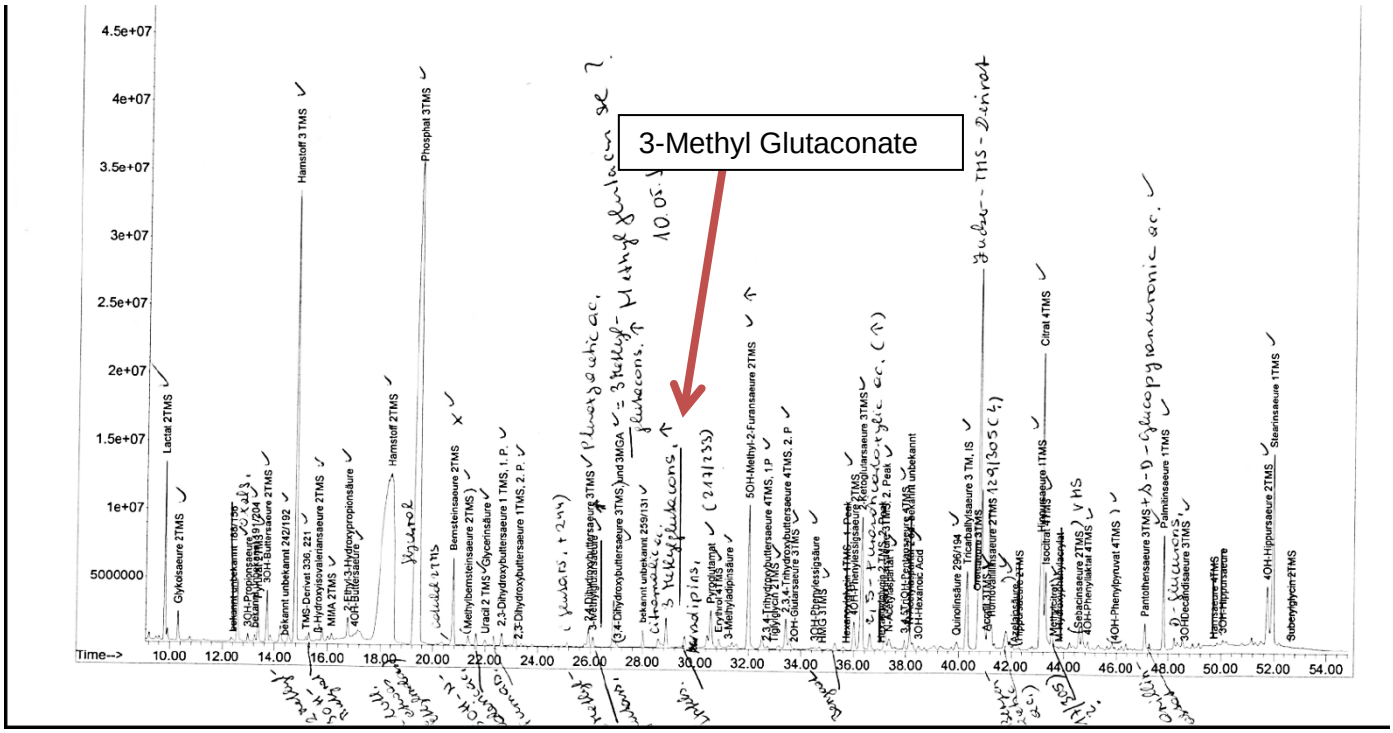
	n	points
3-methylglutaconic acid elevated	13	2

Organic acids column chromatography
3-methylglutaconic acid
n=7
median= 33.00
mean= 28.14
SD= 12.64
min, max= [0.02, 42.00]

Interpretation

	n	Points
3-methylglutaconic aciduria (Barth syndrome or not)	13	2
Barth syndrome	1	1
No disorder	5	0
Pompe disease	1	0

GC_MS TIC sample D



Sample E: Mucopolysaccharidosis type II (Hunter) due to iduronate sulphate sulphatase deficiency (OMIM 309900).

Patient details provided:
A male patient with delayed psychomotor development, macrocephaly without hydrocephalus and restricted joint flexibility. Urine collected on diagnosis at 6y

A 4.5 year old boy, recently arrived in this country, with restricted movement, hepato-splenomegaly and mild mental retardation. 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). This sample was also distributed in 2010.

Analytical performance*:* Elevated total GAG scored one point (18/19 labs). Differentiation of GAGs pointing to a diagnosis including MPS II scored an additional point (16/19 labs)

Interpretative proficiency: A diagnosis of MPS including mention of MPSII scored two points (18 labs) while an incorrect MPS disorder scored one point (one lab).

Overall impression: High overall proficiency of 92% with a clear improvement in performance compared with that previously seen with the same sample.

Analytical Details

Creatinine
n=18 (one value 0.033)
median= 4.03
mean= 4.11
SD= 0.16
min, max= [3.60, 5.39]

pH
n=10
median= 6.00
mean= 6.05
SD= 0.08
min, max= [5.50, 6.50]

Spot tests
All negative

GAG (n= 20)

	n	points
GAG increased	18	1
Differentiation, dermatan/heparan sulphate	16	1

Glycosaminoglycans quantitative
n=16
median= 43.90
mean= 41.67
SD= 209.61
min, max= [4.20, 61.90]

GAG fractionation

Dermatan Sulphate / Heparan Sulphate	12
Dermatan Sulphate	4
Not Done	3

Interpretation

	n	Points
MPS, type II mentioned	18	2
MPS disorder	1	1

Sample F: Dibasic amino aciduria due to lysinuric protein intolerance (OMIM 222700). Gene defect SLC7A7

For full report on the findings in this common sample please go to
http://www.erndim.org/store/docs/Commonsample2013LPIfinal-VEFUCAVA461488-12-4-2013.pdf

Patient details provided:
A 4 year old boy with splenomegaly (known since 6 months of life), failure to thrive and special eating behaviour. The sample was collected at the age of 17 years during a routine check-up while receiving specific treatment

Diagnosis confirmed by mutation analysis

Analytical performance*:* 1 point was given for the finding of dibasic amino aciduria (18 labs) and one point for finding elevated orotic acid (17 labs.)

Interpretative proficiency: The diagnosis of lysinuric protein intolerance scored 2 points (17 Labs), urea cycle disorder, e.g. arginase deficiency + other urea cycle disorders (1 point), OTC deficiency (0 points)

Overall impression:
Good overall performance of 93% with a relatively straightforward sample.

Analytical Details

Creatinine
n=18 (one value 0.046)
median= 4.95
mean= 4.96
SD= 0.07
min, max= [4.49, 5.30]

pH
n=11
median= 7.50
mean= 7.22
SD= 0.51
min, max= [6.00, 8.00]

Spot tests
All negative

Amino acid analysis

	n	points
Dibasic amino aciduria	18	1

Arginine
n=18
median= 86.50
mean= 120.01
SD= 168.41
min, max= [0.08, 808.00]

Lysine
n=18
median= 396.00
mean= 515.27
SD= 651.41
min, max= [0.33, 3167.00]

Ornithine
n=16
median= 14.00
mean= 21.76
SD= 29.27
min, max= [10.94, 135.00]

Citrulline (elevated)
n=7
median= 9.10
mean= 9.26
SD= 4.27
min, max= [0, 13.00]

Orotic acid analysis

	n	points
Orotic acid elevated	17	1

Organic acids column chromatography/**orotic acid**
n=4
median= 69.50
mean= 74.50
SD= 21.43
min, max= [51.00, 108.00]

Orotic acid
n=12
median= 57.00
mean= 58.12
SD= 375.24
min, max= [39.70, 108.00]

Interpretation

	n	Points
LPI.	17	2
Urea cycle disorder, e.g. arginase deficiency	1	1
OTC deficiency	1	0

8. Scores

Overall proficiency

Sample	Diagnosis	A (%)	I (%)	total (%)
A	Isolated methylmalonic aciduria due to the cblA defect (OMIM 251100)	100	84	92
B	Citrullinaemia due to argininosuccinate synthetase deficiency	100	95	97
C	GM1 gangliosidosis (OMIM 230500) due to B-galactosidase (EC 3.2.1.23) deficiency	55	63	59
D	3-Methylglutaconic aciduria in association with Barth Syndrome (OMIM 302060).	68	71	70
E	Mucopolysaccharidosis type II (Hunter)due to iduronate sulphate sulphatase deficiency (	87	97	92
F	Dibasic amino aciduria due to lysinuric protein intolerance (OMIM 222700). Gene defect SLC7A7	95	92	93

Total scores

	Survey 1			Survey 2			
Lab No	A	B	C	D	E	F	Total
1	3	4	4	0	4	4	19
2	3	4	3	0	4	4	18
3	4	2	0	4	4	4	18
4	4	4	4	4	4	4	24
5	3	4	4	4	4	4	23
6	4	4	0	1	4	4	17
7	3	4	3	4	3	1	18
8	4	4	4	4	4	4	24
9	4	4	4	4	4	3	23
10	3	4	0	4	4	3	18
11	4	4	3	4	4	4	23
12	4	4	4	0	4	4	20
13	4	4	4	0	4	4	20
14	4	4	0	0	3	4	17
15	4	4	0	4	4	4	20
16	4	4	0	4	3	4	19
17	4	4	0	4	1	4	17
18	3	4	4	4	4	4	23
19	4	4	4	4	4	4	24

*This year the scores proposed by us were evaluated by a second advisor and confirmed at the Scientific Advisory Board meeting in March 2014. At this meeting the cut off point for satisfactory performance was set at 14 points. Labs failing to reach this mark will receive a performance advice letter.

Detailed Scores: A,B,C

Lab no	Sample A MMAuria			Sample B Citrullinaemia			Sample C GM1 gangliosidosis			
	A	I	Total	A*	I	Total	A	I	Total	Total
1	2	1	3	2	2	4	2	2	4	11
2	2	1	3	2	2	4	1	2	3	10
3	2	2	4	2	0	2	0	0	0	6
4	2	2	4	2	2	4	2	2	4	12
5	2	1	3	2	2	4	2	2	4	11
6	2	2	4	2	2	4	0	0	0	8
7	2	1	3	2	2	4	1	2	3	10
8	2	2	4	2	2	4	2	2	4	12
9	2	2	4	2	2	4	2	2	4	12
10	2	1	3	2	2	4	0	0	0	7
11	2	2	4	2	2	4	1	2	3	11
12	2	2	4	2	2	4	2	2	4	12
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	0	0	0	8
15	2	2	4	2	2	4	0	0	0	8
16	2	2	4	2	2	4	0	0	0	8
17	2	2	4	2	2	4	0	0	0	8
18	2	1	3	2	2	4	2	2	4	11
19	2	2	4	2	2	4	2	2	4	12
ratio	38/38	32/38	70/76	38/38	36/38	74/76	21/38	24/38	45/76	
%	100	84	92	100	95	97	55	63	59	

Detailed Scores: D,E,F

Lab no	Sample D 3-Methylglutaconic aciduria			Sample E MPS II			Sample F Lysinuric Protein intolerance			
	A	I	Total	A*	I	Total	A	I	Total	Total
1	0	0	0	2	2	4	2	2	4	8
2	0	0	0	2	2	4	2	2	4	8
3	2	2	4	2	2	4	2	2	4	12
4	2	2	4	2	2	4	2	2	4	12
5	2	2	4	2	2	4	2	2	4	12
6	0	1	1	2	2	4	2	2	4	9
7	2	2	4	1	2	3	1	0	1	8
8	2	2	4	2	2	4	2	2	4	12
9	2	2	4	2	2	4	2	1	3	11
10	2	2	4	2	2	4	1	2	3	11
11	2	2	4	2	2	4	2	2	4	12
12	0	0	0	2	2	4	2	2	4	8
13	0	0	0	2	2	4	2	2	4	8
14	0	0	0	1	2	3	2	2	4	7
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	1	2	3	2	2	4	11
17	2	2	4	0	1	1	2	2	4	9
18	2	2	4	2	2	4	2	2	4	12
19	2	2	4	2	2	4	2	2	4	12
ratio	26/38	27/38	53/76	33/38	37/38	70/76	36/38	35/38	71/76	
%	68	71	70	87	97	92	95	92	93	

9. 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. The level for satisfactory performance for this year will be set at the SAB meeting in November. A special meeting of scientific advisors took place in November 2012 to consider how to harmonise scoring within all our qualitative schemes and the question of introducing critical errors in our schemes. Here it was decided to incorporate recommendations into interpretation giving a 2 plus 2 scoring system. Also the concept of **critical error** was accepted to be introduced in 2013. Thus labs failing to make a correct diagnosis of a sample considered as eligible for this category will be deemed not to have reached a satisfactory performance even if their total points for the year exceed the limit set at the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on March 20, 2014 although it was decided to postpone actual scoring of critical error until 2014. Thus samples A, B, E, F would have been considered straightforward and a failure to recognise an abnormality would have been scored as a critical error leading to unsatisfactory performance regardless of the total points scored.

10. Annual meeting

The annual meeting of participants of the 5 DPT centres takes place during the SSIEM symposium in Innsbruck on Tuesday, September 2nd, 9.00.

11. Changes planned for 2014

No changes are envisaged

12. Tentative schedule and fee in 2014

Sample distribution	07 April 2014
Start of analysis of Survey 2014/1 Website open	April 28
Survey 2014/1 - Results submission	May 19
Survey 2014/1 - Reports	June 21
Start of analysis of Survey 2014/2	June 9
Survey 2014/2 – Results submission	June 30
Survey 2014/2 - Reports	August 7
Annual meeting of participants	Sept 2 Innsbruck SSIEM
Annual Report 2014	April 2015

13. ERNDIM certificate of participation

A combined certificate of participation covering all EQA schemes will be provided to all participants who take part in any ERNDIM scheme. For the DPT scheme this certificate will indicate if results were submitted and whether satisfactory performance was achieved in the scheme.

Basel, April 2014

Brian Fowler
Scientific advisor