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Diagnostic Proficiency Testing

Survey 2015

Final Report

prepared by
Brian Fowler

1. Geographical distribution of participants

In 2015, 21 laboratories from 10 countries subscribed to the scheme. Nineteen laboratories submitted results for the first sample batch and twenty one labs for the second batch.

Country	Number of participants
Australia	2
Austria	1
Canada	3
China	1
Estonia	1
Germany	3
Netherlands	1
Norway	1
Sweden	2
Switzerland	1
USA	5

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 7th at ambient temperature by CSCQ using the courier DHL. Delivery times of samples reported by the courier were all within a few days and these discrepancies with suggested possible internal delays within the institution.

3. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/ pyrimidines were required in 2015.

4. Schedule of the scheme in 2015

Sample distribution	April 07, 2015
Start of analysis of Survey 2015/1	April 27, 2015
Survey 2015/1 - Results submission	May 18, 2015
Survey 2015/1 - Reports on website	June 23, 2015
Start of analysis of Survey 2015/2	June 08, 2015
Survey 2015/2 – Results submission	June 29, 2015
Survey 2015/2 - Reports on website	August 10, 2015
Annual meeting of participants	SSIEM, Lyon, September 1, 2015
Annual Report 2015	April 2016

This year we were able to use the evaluation programme to generate individual lab reports and these were made available on the CSCQ website in good time on June 23rd and August 10th. Feedback on the content and style of these reports is invited.

5. Receipt of samples and results

Receipt of samples (sent on April 07, 2015)

Time after shipment	Delivery reported by courier	Delivery reported by participants
1 day	9	3
2 days	9	7
3 days	1	3
6-7 days	2	1
20-21 days		5
3rd April !!		1

Discrepancies must be due to delays within the institution.

Date of reporting of results

19 / 21 labs returned results for the first and second surveys respectively and 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 may be considered by the scheme organisers in evaluating interpretation.

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: **Odontohypophosphatasia OMIM #241510**

Patient details provided
17 year old female, currently asymptomatic but with a history of dental problems in childhood

Further information
The sample came from the University Children's Hospital, Zürich. This child and a sibling presented in early childhood with only dental symptoms and they have remained mono-symptomatic. They carry mutations in the *ALPL* gene.

Analytical performance:
10 of 19 labs reported a value for phosphoethanolamine. Two labs reported this to be normal but nevertheless found quantitative values close to the mean for all labs. Therefore provisionally this was scored as two points.

Interpretative proficiency
Of the ten labs reporting phophoethanolamine 2 stated that this was normal, 8 as elevated. These eight labs correctly diagnosed hypophosphatasia. This points to the need to carefully consider **AGE related reference values** within and between laboratories.

Overall impression:

This was a fairly difficult sample due to the only mild elevation of phophoethanolamine. Nevertheless 10 labs correctly quantified this and 8 gave the correct diagnosis.
This was considered by the SAB to be an educational sample, not valid for critical error and scores were not included for assessing overall performance.

Analytical Details

Creatinine
n=19
median= 9.12
mean= 9.14
SD= 0.30
min, max= [8.36, 10.38]

pH
n=10
median= 6.25
mean= 6.45
SD= 0.24
min, max= [6.00, 7.00]

Spot tests
All normal except for one trace of sulphocysteine

Amino Acid Analysis

	n	points
Phospho-ethanolamine increased	8	2
Phospho-ethanolamine measured correctly but not called elevated	2	2

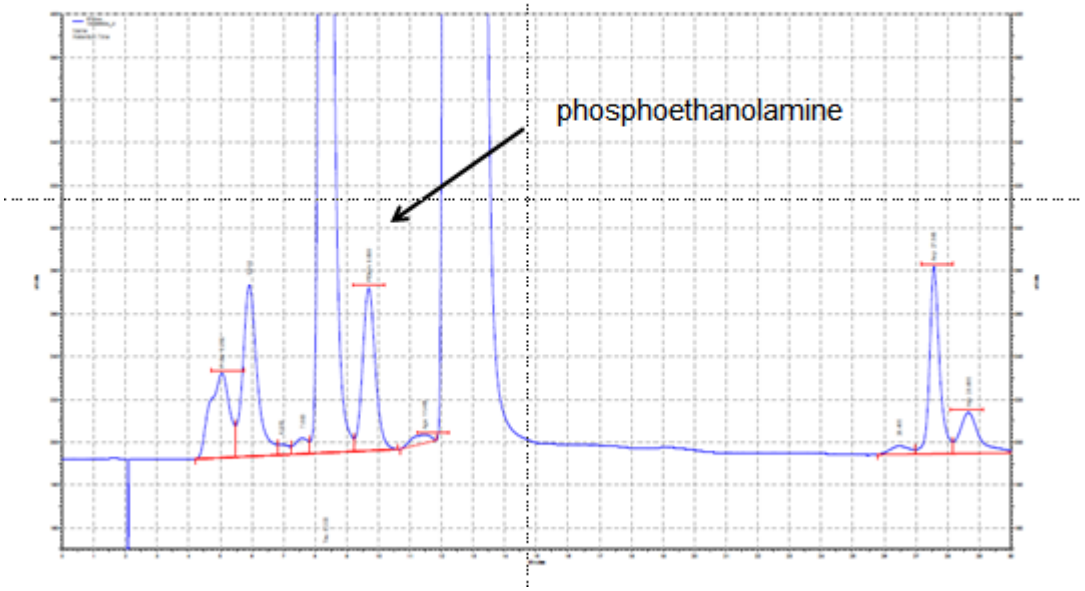
Phospho-ethanolamine
n=9
median= 17.20
mean= 31.18
SD= 41.32
min, max= [14.00, 148.00]

Interpretation

	n	Points
Hypophosphatasia	8	2
Hypophosphatasia mentioned in recommendations	2	1
No diagnosis/Normal	8	0
Non-ketotic hyperglycinaemia (asymptomatic)	1	0

AA chromatogram

Ion-exchange chromatogram (courtesy M. Hersberger, Zürich Children's Hospital)



Sample B: Sialidosis due to neurominidase deficiency, OMIM #256550

Patient details provided:
Small for dates boy with history of complications in pregnancy developed necrotising enterocolitis, liver and spleen enlargement and died at 2 months of age. Urine collected over several days without any treatment.

Further information
This sample was provided by Sabine Scholl from Innsbruck. Hydrops fetalis was found leading to prenatal investigations revealing intrauterine hepatosplenomegaly.
The child born was born prematurely at 35 weeks with a birth weight of 2300 g. and subsequently developed necrotising enterocolitis, increasing hepatomegaly and feeding difficulties and died at two months of age.
Sialidosis was confirmed by oligosaccharide analysis, enzyme assay and mutation analysis.

Analytical performance*:*
Eleven labs reported on tlc for oligosaccharides, 10 reported an abnormal pattern (2 points), one lab mentioned unclear bands (one point). Three labs measured sialic acid, two finding elevated, one normal level*.*

Interpretative proficiency:
Seven labs made a diagnosis of sialidosis based on the oligosaccharide pattern (2 points) whilst four labs thought the pattern indicated gm1-gangliosidosis (1 point). Three labs recommended performance of oligosaccharide analysis (1 point)..

Overall impression
Intermediate proficiency of a straightforward sample necessitating oligosaccharide analysis with differences in interpretation of the pattern.
This was considered by the SAB to be an educational sample, not valid for critical error and scores were not included for assessing overall performance

Analytical Details

Creatinine (1 outlier)
n=18
median= 0.45
mean= 0.47
SD= 0
min, max= [0.40, 0.65]

pH
n=10
median= 8.75
mean= 8.50
SD= 0.33
min, max= [7.50, 9.00]

Spot tests
All negative

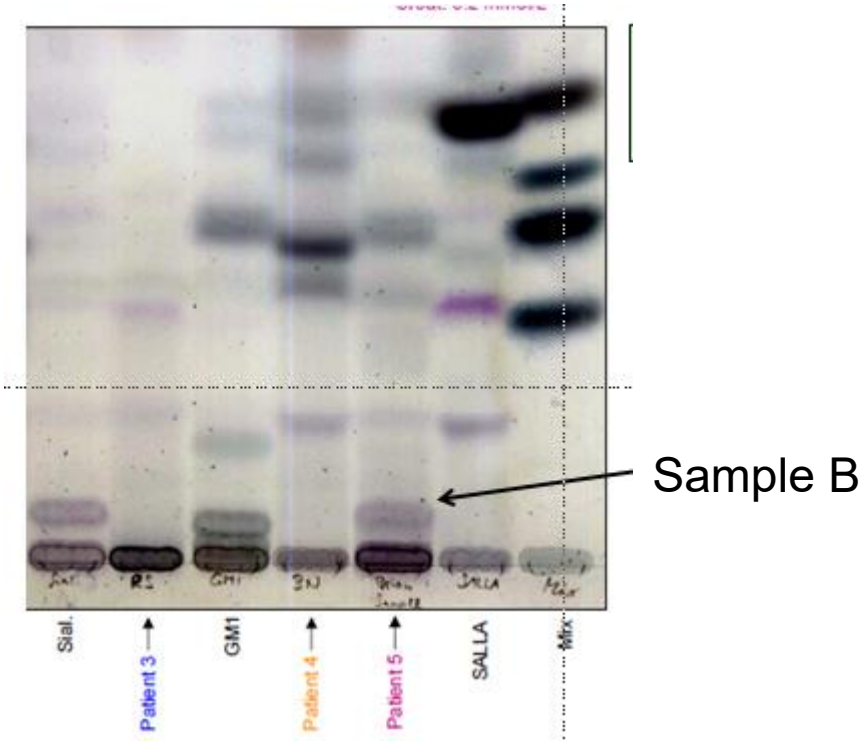
Oligosaccharide analysis (n=11)

	n	points
Abnormal pattern	10	2
Unclear finding	1	1

Interpretation

	n	Points
Sialidosis	7	2
GM1 gangliosidosis	4	1
Recommendation to perform oligosaccharide analysis	3	1

Oligosaccharide TLC
(courtesy P. Burda, Zürich)



Sample C: Homocystinuria /Methylmalonic aciduria due to the cblC defect OMIM #277400

Patient details provided:
A male child presenting with failure to thrive and delayed psychomotor development. EEG abnormalities and hypotonia were observed on examination. Urine was collected at 5 years of age while receiving specific treatment.

Further information
The sample came from the University Children's Hospital Zürich and had previously been distributed in 2011. The patient was admitted at 26 days of age to the emergency department following a history of sleepiness and feeding difficulties. He presented with severe breathing difficulties, recurrent cyanosis followed by respiratory arrest. He recovered after intubation but showed liver and kidney dysfunction, convulsions, muscular hypotonia, lethargy and pancytopenia. He responded rapidly to treatment. The patient has the cblC defect confirmed by fibroblast studies of propionate fixation, methionine synthesis and cobalamin coenzyme uptake in fibroblasts and mutation analysis.

Analytical performance*:*

17 of 19 labs reported elevated or slightly elevated methylmalonic acid (1 point). Ten labs reported an amino acid abnormality (elevated cystathionine or homocysteine, low methionine) indicative of the diagnosis (1 point).

Interpretative proficiency:
Mention of a combined MMA/HC defect as definite or differential diagnosis, or recommendations for appropriate follow up of MMAuria was considered to be correct (2 points). Mention of only an isolated MMA defect was scored with one point.

Overall impression

A fairly difficult sample pointing to the wide differential of elevated MMA. A sample from this patient had been circulated in 2011. This time the urine was very dilute but still distributed to test labs proficiency with such low but nevertheless abnormal metabolite levels.
Regarding critical error, it was considered classifying missing MMA as critical error but this was rejected due to the very dilute sample.

Analytical Details

Creatinine (1 outlier)
n=18
median= 0.30
mean= 0.30
SD= 0
min, max= [0.22, 0.55]

pH
n=10
median= 9.00
mean= 8.85
SD= 0.11
min, max= [8.00, 9.00]

Spot tests

All negative

Organic acid analysis (n= 19)

Organic acids screening
Abnormal profile
n=4

Organic acids column chromatography
methylmalonic acid
n=11
median= 105.00
mean= 105.41
SD= 41.85
min, max= [11.00, 154.00]

	n	points
Methylmalonic acid elevated	17	1
Methylcitric acid	3	-

Amino acid analysis (n= 10)

Amino acid quantitative
cystathionine
n=5
median= 60.00
mean= 74.88
SD= 32.88
min, max= [49.10, 139.40]

Amino acid quantitative
methionine
n=5
median= 7.10
mean= 6.69
SD= 6.72
min, max= [0.00, 18.60]
VALUES 0.45, 7.1, 7.3,18.6

Amino acid quantitative
Homocysteine
n=3
median= 7.00
mean= 24.67
SD= 30.06
min, max= [0.01, 67.00]
VALUES 0.01, 7.0, 67

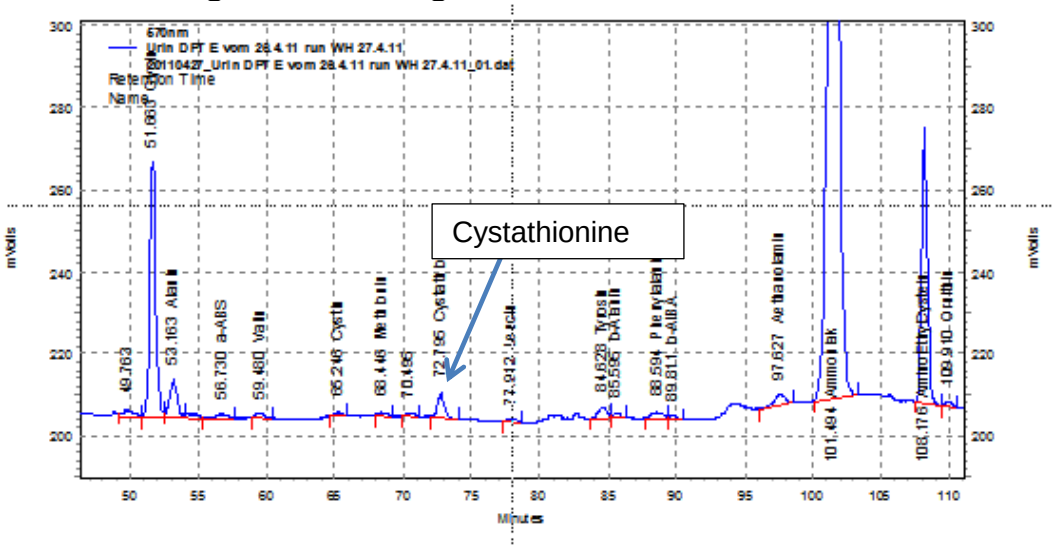
Homocyst(e)ine
n=2
median= 21.48
mean= 21.48
SD= 841.52
min, max= [0.97, 42.00]

	n	points
Homocyst(e)ine elevated	2	1
Methionine low	3	1
Methionine elevated	2	0
Cystathionine elevated	6	1

Interpretation

	n	Points
Methylmalonic aciduria including cblC	13	2
Methylmalonic aciduria not including cblC	2	1
Other diagnosis/	4	0

Ion-Exchange chromatogram



Sample D: Homocystinuria due to cystathionine beta-synthase deficiency, OMIM #236200

Patient details provided:
34 year-old woman, with normal psychomotor development, investigated because of phlebitis at the age of 30 (under oestrogens).

Further information
This was the common sample and full details were presented at the ERNDIM workshop of 2015 in Lyon following the participants meeting.

Analytical performance: The finding of elevated homocyst(e)ine (19 / 21 labs) and elevated methionine (11/21 labs) was key to allowing a specific diagnosis. Three labs reported values for methionine of 16, 20 and 22.05 mmol/mol, close to the median of 18.30 but interpreted as normal pointing to reference values as a possible issue.
The consensus of all DPT centres was to award two points for finding elevated homocyst(e)ine and missing this was judged by the SAB to be a critical error. The finding of elevated methionine was not scored but in some cases this influenced the interpretation.

Interpretative proficiency:

14/21 labs gave CBS deficiency as the most likely diagnosis (2 points) and two labs mentioned an unspecified type of homocystinuria (one point). Other diagnoses scored with zero points were MTHFR deficiency without mentioning CBS deficiency (three labs), methylhydroxy butyric aciduria (one lab) and sialidosis/MAT deficiency (one lab).

Overall impression:
Fairly good proficiency was seen in detecting homocystinuria but less good for the differential diagnosis which hinged on elevated and not low methionine.

Analytical Details

Creatinine
n=21
median= 4.41
mean= 4.49
SD= 0.05
min, max= [4.24, 5.00]

pH
n=10
median= 6.00
mean= 5.95
SD= 0.13
min, max= [5.00, 6.50]

Spot tests

All negative

Amino acid analysis (n= 21)

	n	points
Homocystine elevated	19	2
Methionine elevated	11	(1)
Methionine normal/low	3	0
Amino acids – no abnormality	1	0

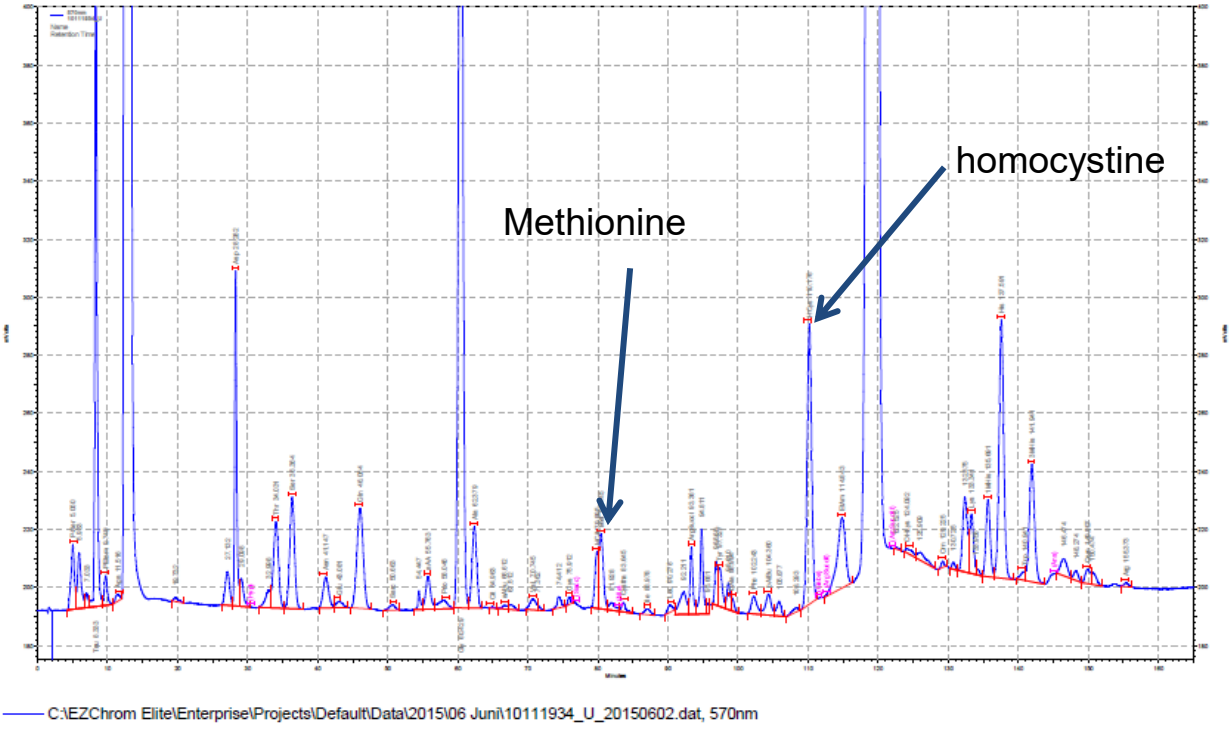
Amino acid quantitative
methionine
n=13
median= 18.30
mean= 26.31
SD= 30.98
min, max= [12.00, 133.00]

Amino acid quantitative
homocystine
n=18
median= 36.00
mean= 105.22
SD= 189.25
min, max= [18.00, 829.00]

Interpretation

	n	Points
CBS deficiency homocystinuria	14	2
Unspecified homocystinuria	2	1
MTHFR deficiency	3	0
Other diagnosis	2	0

AA ion-exchange chromatogram



Sample E: Dihydropyriminidase deficiency; OMIM #222748 (DPYSD gene)

Patient details provided:
A 6 year old boy who came to attention because of intermittent macro-haematuria. The sample was collected during a symptom free interval with no treatment.

Further information
The sample was provided by Sabine Scholl, Innsbruck . The patient has not developed any other symptoms than macrohaematuria. The diagnosis was confirmed by mutation analysis.

Analytical performance*:* 17 labs reported pyrimidine abnormalities (2 points).

Interpretative proficiency:
15 labs reported dihydropyriminidase and/or dihydropyrimidine dehydrogenase deficiency (2 points).
One point was given for the recommendation of pyrimidine / purine analysis in the absence of analyte abnormalities (3 labs).

Overall impression:
The finding of the dihydro- forms of the metabolites allows the specific diagnosis of dihydropyriminidase deficiency and not dihydropyrimidine dehydrogenase deficiency. However following discussion at the participants meeting and the SAB it was agreed to score 2 points for analytical performance for detection of any pyrimidine abnormality and two points for diagnosis of dihydropyriminidase deficiency and/or dihydropyrimidine dehydrogenase deficiency.
This sample was considered by the SAB not to be valid for critical error.

Analytical Details

Creatinine
n=21
median= 2.91
mean= 2.93
SD= 0.09
min, max= [2.11, 3.84]

pH
n=11
median= 6.00
mean= 6.27
SD= 0.11
min, max= [6.00, 7.00]

Spot tests
All negative

Organic acid screening
Normal profile: 1
Borderline: 1
Abnormal profile: .4

	n	points
Thymine and/or Uracil by Organic acid or P & P analysis	17	2
No abnormality	4	0
Dihydro-thymine / Dihydro-uracil Organic acids	4	-
Dihydro-thymine / Dihydro-uracil P&P anal.	4	-

Quantitative

Organic acids column chromatography

	N	median	mean	S.D.	Range
Thymine	3	70	70.3	7.8	61-80
Uracil	3	45	47	9.9	36-60
Dihydro-thymine	1	30			
Dihydro-uracil	1	296			

Purine / Pyrimdine analysis					
	N	median	mean	S.D.	Range
Thymine	4	8.5	8.8	1.4	7 - 11
Uracil	5	22	27	8	18 - 37
Dihydro-thymine	2				114/189
Dihydro-uracil	2				139 - 215

Interpretation

	n	Points
Dihydropyriminidase deficiency	7	2
Dihydropyrimidine dehydrogenase deficiency	3	2
DHP / DHPdeH	5	2
Purine / Pyrimidine analysis recommended	3	1
No diagnosis	3	0

GCMS and spectra of dihydrouracil and dihydrothymine available on request.

Sample F: Glutaric acidaemia type I due to glutaryl-CoA dehydrogenase deficiency OMIM #231670, GCDH gene

Patient details provided:
This boy was investigated for progressive macrocephaly at age 6 months. The urine sample was collected at age 3 y, while receiving specific therapy.
.
Further information
The sample was provided by George Ruijter. In fact this patient was detected by newborn screening and has remained asymptomatic. 3-OH-glutaric acid in urine and C5DC in plasma were markedly elevated.

Analytical performance*:*
Key to the diagnosis is the finding of elevated 3-hydroxyglutaric acid. Glutaric acid was not consistently found to be elevated (5 elevated /4 normal) but there was overlap of values evaluated as normal or elevated within the quantitative range of 4.0 - 29.0 mmol/mol (n=8)

Interpretative proficiency:
19/21 labs made the correct diagnosis of GAI with a possible MPSIV and Urea cycle reported by the other two labs..

Overall impression:
This is a fairly difficult sample due to the low glutarate but the clear elevation of 3-hydroxy glutarate found by 19 labs reflects overall good performance.
Failure to detect 3-hydroxyglutaric acid was judged by the SAB to be a critical error.

Analytical Details

Creatinine
n=21
median= 2.79
mean= 2.78
SD= 0.01
min, max= [2.64, 3.03]

pH
n=10
median= 7.75
mean= 7.60
SD= 0.21
min, max= [7.00, 8.00]

Spot tests
All negative

Organic acid screening
Normal profile: 0
Borderline: 0
Abnormal profile: 6

Organic acid analysis

	n	points
3-Hydroxyglutaric acid elevated	20	2
Glutaric acid elevated	5	0
Glutaric acid normal	4	0

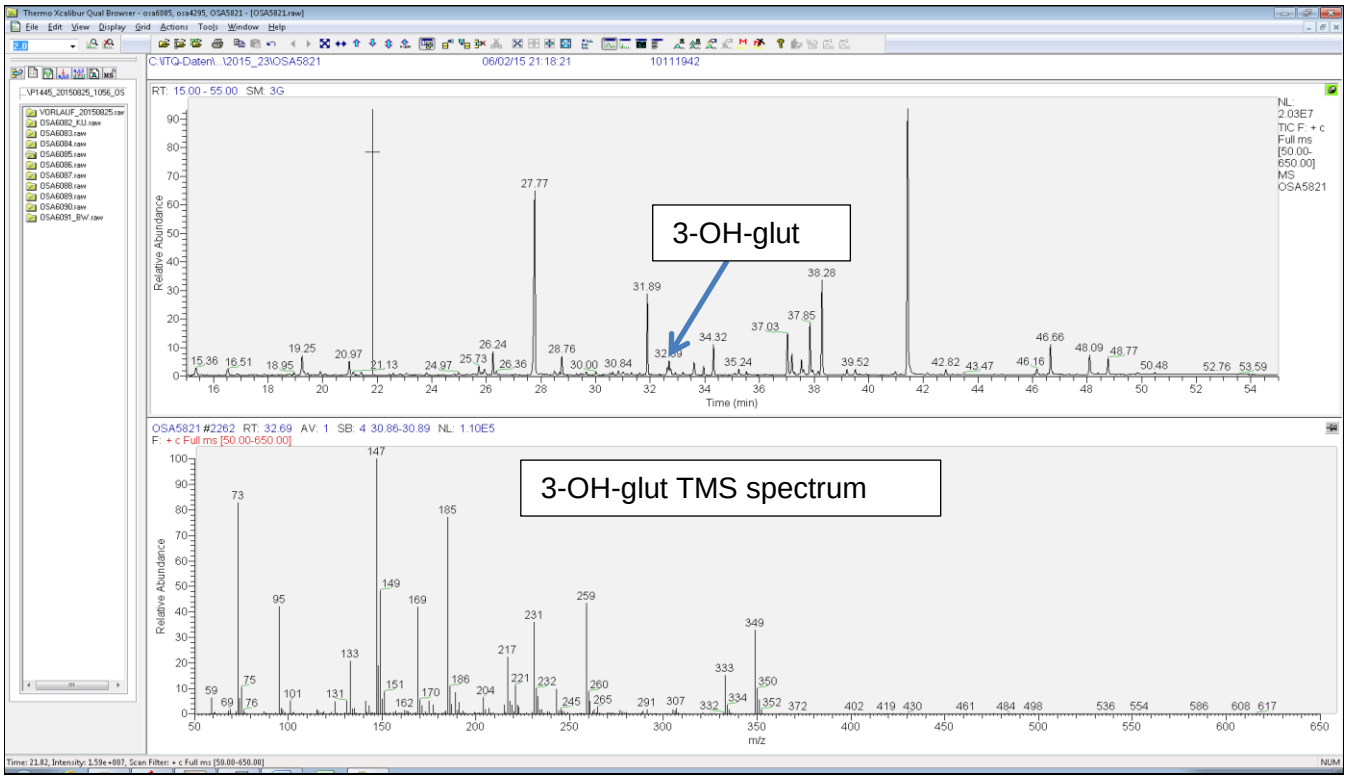
Organic acids column chromatography **glutaric acid** n=8 median= 5.70 mean= 9.45 SD= 8.00 min, max= [4.00, 29.00]

Organic acids column chromatography **3-hydroxyglutaric acid** n=10 median= 31.50 mean= 33.86 SD= 16.22 min, max= [12.30, 61.00]

Interpretation

	n	Points
Glutaric aciduria GA1	19	2
Other diagnosis	2	0

GCMS Chromatogram



8. Scores

Overall proficiency

Sample	Diagnosis	A (%)	I (%)	total (%)
A	Odontohypophosphatasia OMIM #241510	58	45	51
B	Sialidosis due to neuraminidase deficiency, OMIM #256550	55	55	55
C	Homocystinuria /Methylmalonic aciduria due to the cblC defect OMIM #277400	71	74	72
D	Homocystinuria due to cystathionine beta-synthase deficiency, OMIM #236200	93	78	88
E	Dihydropyriminidase deficiency; OMIM #222748, *DPYSD* gene	81	79	80
F	Glutaric acidaemia type I due to glutaryl-CoA dehydrogenase deficiency OMIM #231670, *GCDH* gene	90	90	90

Total scores

	Survey 1			Survey 2				
Lab No	**A**	**B**	**C**	**D**	**E**	**F**	**Total**	**Total – A & B**
1	1	4	4	4	2	4	**19**	**14**
2	0	0	3	1	4	0	**8**	**8**
3	0	0	4	4	4	4	**16**	**16**
4	-	-	-	2	4	4	**10***	**10**
5	4	0	4	4	1	4	**17**	**13**
6	0	3	0	4	4	4	**15**	**12**
7	4	0	4	4	0	4	**16**	**12**
8	4	4	3	4	4	4	**23**	**15**
9	4	2	4	4	4	4	**22**	**16**
10	2	1	4	0	1	0	**8**	**5**
11	2	4	3	4	4	4	**21**	**15**
12	4	0	0	2	3	4	**13**	**9**
13	4	1	4	4	4	4	**21**	**16**
14	4	4	3	4	4	4	**23**	**15**
15	-	-	-	4	4	4	**12***	**12**
16	2	1	4	4	0	4	**15**	**12**
17	0	3	1	2	4	4	**14**	**11**
18	4	4	3	4	4	4	**23**	**15**
19	0	4	4	3	4	4	**19**	**15**
20	0	4	2	4	4	4	**18**	**14**
21	0	3	1	4	4	4	**16**	**13**

The scores proposed by us were evaluated by a second advisor and confirmed at the Scientific Advisory Board meeting in March 2016. At this meeting the cut off point for satisfactory performance was set (see below). Labs failing to reach this mark will receive a performance advice letter.

Detailed Scores: A,B,C

Lab no	Sample A Hypophosphatasia			Sample B Sialidosis			Sample C cblC			
	A	I	Total	A	I	Total	A	I	Total	Total
1	1	0	1	2	2	4	2	2	4	**9**
2	0	0	0	0	0	0	2	1	3	**3**
3	0	0	0	0	0	0	2	2	4	**4**
4	-	-	-	-	-	-	-	-	-	
5	2	2	4	0	0	0	2	2	4	**8**
6	0	0	0	2	1	3	0	0	0	**3**
7	2	2	4	0	0	0	2	2	4	**8**
8	2	2	4	2	2	4	1	2	3	**11**
9	2	2	4	1	1	2	2	2	4	**10**
10	1	1	2	0	1	1	2	2	4	**7**
11	2	0	2	2	2	4	1	2	3	**9**
12	2	2	4	0	0	0	0	0	0	**4**
13	2	2	4	0	1	1	2	2	4	**9**
14	2	2	4	2	2	4	1	2	3	**11**
15	-	-	-	-	-	-	-	-	-	
16	2	0	2	0	1	1	2	2	4	**7**
17	0	0	0	2	1	3	1	0	1	**4**
18	2	2	4	2	2	4	1	2	3	**11**
19	0	0	0	2	2	4	2	2	4	**8**
20	0	0	0	2	2	4	1	1	2	**6**
21	0	0	0	2	1	3	1	0	1	**4**
ratio	22/38	17/38	**39/76**	21/38	21/38	**42/76**	27/38	28/38	**55/76**	
%	58	45	51	55	55	55	71	74	72	

Detailed Scores: D,E,F

Lab no	Sample D CBS def.			Sample E Pyrimindase def.			Sample F GA1			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	0	2	2	2	4	10
2	1	0	1	2	2	4	0	0	0	5
3	2	2	4	2	2	4	2	2	4	12
4	2	0	2	2	2	4	2	2	4	10
5	2	2	4	0	1	1	2	2	4	9
6	2	2	4	2	2	4	2	2	4	12
7	2	2	4	0	0	0	2	2	4	8
8	2	2	4	2	2	4	2	2	4	12
9	2	2	4	2	2	4	2	2	4	12
10	0	0	0	0	1	1	0	0	0	1
11	2	2	4	2	2	4	2	2	4	12
12	2	0	2	2	1	3	2	2	4	9
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	2	2	4	12
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	0	0	0	2	2	4	8
17	2	2	2	2	2	4	2	2	4	10
18	2	2	4	2	2	4	2	2	4	12
19	2	1	3	2	2	4	2	2	4	11
20	2	2	4	2	2	4	2	2	4	12
21	2	2	4	2	2	4	2	2	4	12
ratio	39/42	33/42	**74/84**	34/42	33/42	**67/84**	38/42	38/42	**76/84**	
%	93	78	88	81	79	80	90	90	90	

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 introduced in **2014.** 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.
This year samples D and F qualified as critical error.
Samples A and B were judged to be educational and their scores not included in assessment of overall performance. Thus the level set for **satisfactory performance is ten points** and below this is evaluated as unsatisfactory.

10. Annual meeting

The annual meeting of participants of the 5 DPT centres took place during the SSIEM symposium in Lyon on Tuesday, September 1st, 09.00. This was attended by 21 participants representing 11 centres.

11. Changes planned for 2016

It was noticed that there is inconsistency between centres in the use of thiomersal as an anti-bacterial agent in addition to heat treatment of urine samples. Since those centres not using thiomersal have not experienced any sample deterioration it was decided to harmonize policy and in the future thiomersal will not be added to samples.
Otherwise no changes are envisaged

12. Tentative schedule and fee in 2016

Sample distribution	February 1st, 2016
Start of analysis of Survey 2016/1	February 22nd, 2016
Survey 2016/1 - Results submission	March 14th, 2016
Survey 2016/1 - Reports	May 15th, 2016
Start of analysis of Survey 2016/2	May 23rd, 2016
Survey 2016/2 – Results submission	June 6th, 2016
Survey 2016/2 - Reports	July 31st, 2016
Annual meeting of participants	SSIEM, Rome, September 6, 2016
Annual Report 2016	April 2017

Fee was set at €396.

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 / Zürich, April 2016

Brian Fowler
Scientific advisor