Department of Clinical Chemistry and Newborn Screening
Mrs C Scott, Scientific Advisor
Professor J R Bonham
Dr Jane Dalley, Deputy Scientific Advisor
Telephone (+)44 114 271 7404
Fax (+)44 114 270 6121

The Children's Hospital
Sheffield
S10 2TH
UK
E-mail: Sheffield_urine_organics_EQA@sch.nhs.uk

5th February 2015

QAP for qualitative urinary organic acid analysis

Annual Report 2014 (Sheffield)

Scheme Design

The scheme has been designed and planned by Mrs C Scott and Dr Jane Dalley as Scientific Advisor/Scheme Organiser and deputy Scientific Advisor/Scheme Organiser, respectively, both appointed by and according to procedures laid down by the ERNDIM Board.

Samples and shipment

All EQA materials are 2 ml of heat-treated urine. All samples are obtained following local ethical and consent guidelines. Three sets of three samples (numbered 214-222) were dispatched together in April 2014. Submission deadlines were 20th June (samples 214-216), 19th September (samples 217-219) and 21st November (samples 220-222).

Participation

Active participants (reporting on at least one set of samples in the year) are shown in Table 1 (page 2). The numbers of participants remain steady. New applicants are distributed between the Sheffield and Heidelberg qualitative urinary organic acid schemes which are run separately. The two organising laboratories each participate in the other's scheme.

Results

Laboratories were asked to analyse the sample sets at intervals during the year as if they were separate circulations. Ninety-one laboratories returned results for all three sets; three returned only two, three laboratories made only a single return, and four made no return.

All submitted results are treated as confidential information and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting

Table 1: Geographical distribution of registered participants

	2014	*2013*	*2012*	*2011*	*2010*	*2009*	*2008*
Argentina	3	2	2	2	2	2	1
Australia	6	6	6	6	6	6	6
Belgium	6	6	5	5	6	7	5
Brazil	2	2	2	2	-	1	1
Canada	1	1	1	1	1	1	1
Chile	1	-	-	-	-	-	-
Columbia	1	1	1	1	1	1	-
Czech Republic	-	-	-	1	-	-	-
Democratic Republic of China	1	1	1	2	2	1	1
Finland	2	2	1	1	1	1	1
France	15	15	15	15	13	13	14
Hong Kong	1	-	-	-	-	-	-
Germany†	1	1	1	1	1	1	1
Israel	3	3	3	3	4	3	2
Japan	2	1	1	1	1	1	1
Kingdom of Saudi Arabia	1	-	-	-	-	-	-
Lebanon	1	1	1	1	1	1	1
Malaysia	3	3	3	3	4	3	3
New Zealand	1	1	1	1	1	1	1
Pakistan	1	-	-	-	-	-	-
People's Republic of China	7	9	8	10	7	7	6
Portugal	2	2	2	2	2	2	2
Republic of Korea	1	1	1	1	1	1	1
Republic of Ireland	1	1	1	1	1	1	1
Republic of Singapore	1	1	1	1	1	1	1
South Africa	2	2	2	2	2	1	1
Spain	6	6	6	6	6	6	5
Turkey	4	3	3	3	3	2	2
United Kingdom	18	18	18	18	19	20	20
USA	5	5	3	3	3	4	4
Uruguay	1	-	-	-	-	-	-
Venezuela	-	-	1	1	1	1	1
Vietnam	1	1	1	1	-	-	-
TOTAL	**101**	**94**	**91**	**95**	**90**	**89**	**83**

† Heidelberg laboratory

Scoring of results

To enable data reduction the results were scored as shown below:

Satisfactory	4	Helpful but incomplete	3
Not helpful	2	Slightly misleading	1
Misleading	0		

One point was deducted for each transposed sample number

For the 2014 scheme onwards another criterion for satisfactory performance will be the absence of any "critical error" which is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient.

Table 2: Distribution of scores for individual samples (laboratories making returns)

Sample	Scores				
	0	**1**	**2**	**3**	**4**
Sample 214 3 year old male with vomiting and mild developmental delay Increased excretion 2-hydroxyisovaleric acid and 2 -hydroxy 3- methylvaleric acid. Most likely diagnosis is Maple syrup Urine Disease MSUD or Intermittent Maple syrup Urine Disease (MSUD). Further analysis plasma amino acids.	9	5	1	-	82
Sample 215 4 year old female on treatment. Originally, diagnosed at 6 days old after presenting unwell with moderate hyperammonaemia. Significantly increased excretion of hippurate and benzoate secondary to treatment for hyperammonaemia. Slightly increased excretion of orotic acid along with a moderate peak of a cyclic derivative of citrulline indicates a urea cycle defect, the most likely diagnosis being Citrullinaemia. To investigate further, suggest check plasma amino acids.	6	1	1	2	87
Sample 216 6 year old boy with global developmental delay No abnormality was detected.	2	-	-	-	96
Sample 217 4 year old female with moderate developmental delay Increased excretion of 3-methylglutarate and 3-methylglutaconic acid. Which is indicative of a 3-methylglutaconic aciduria. With the absence of an increased 3-hydroxyisovaleric acid, clinical history and gender of the patient secondary 3-methylglutaconic aciduria would be most likely.	1	1	0	10	80
Sample 218 7 year old female with autism. Nothing specifically diagnostic.	1	0	0	1	90
Sample 219 60 year old female, unknown myopathy, on treatment. Increased excretion of homovanillic acid (HVA) and vanilyllactate. Results would be consistent with treatment with L-dopa. If the patient is not on L-dopa suggest further investigation.	8	3	8	13	60
Sample 220 64 year old male, extreme thirst, intellectual disability Increased excretion of phenylacetic acid, 2-hydroxyphenylacetic, mandelic, phenyllactic acid, 4 hydroxyphenylacetic acid and 4 hydroxyphenyllactic acid. These findings indicate a disorder of phenylalanine catabolism, either in phenylalanine hydroxylase itself (phenylketonuria) or the supply of its tetrahydrobiopterin cofactor. The large peak due to benzoic acid and the absence of phenylpyruvic acid suggests sample deterioration	2	-	2	-	88
Sample 221 24 year old female diagnosed with metabolic defect in infancy, on riboflavin. Presented unwell following short illness Markedly increased excretion of lactate. Also increased excretion of 3 hydroxybutyrate with only a mild increase of acetoactete. Increased excretion of glutarate, fumarate and malate also detected. No increase acylglycines detected. Results possibly indicative of a riboflavin responsive glutaric aciduria type 3 or glutaric aciduria type 2 (GA2). A disorder of mitochondrial function is also a possibility. Further investigations are required; including acylcarnitines, fibroblast fatty acid oxidation flux studies and mutational analysis. (A muscle biopsy might also be considered). [Paracetamol, ibuprofen and antibiotic metabolites detected].	1	-	4	17	70
Sample 222 5 year old male with developmental delay Nothing specifically diagnostic.	2	-	-	-	90

Table 3: Cumulative scores for 2012 - 2014 (current Sheffield participants only)

The maximum annual scores for 2012 were 18. The maximum scores for 2013 & 2014 were 36. An average score per case has not been provided in this annual report due to the new scoring system. An average score as a percentage of the maximum score achievable over the past 3 years has been provided when there have been 3 returns for 3 consecutive years.

Lab Number	No of returns 2014	Total score 2014 (out of 36 for 3 returns)	Number of returns 2013	Total score 2013 (out of 36 for 3 returns)	Number of returns 2012	Total score 2012 (out of 18 for 3 returns)	Average score as a percentage over 3 years*.
2	2	16	3	32	-	-	**-**
3	3	35	3	34	3	18	**97**
4	3	36	3	36	3	18	**100**
5	3	36	3	36	3	18	**100**
6	3	35	3	36	3	18	**99**
7	3	32	3	36	-	-	**-**
10	3	36	3	34	3	14	**90**
11	2	24	3	36	3	18	**-**
12	3	36	3	36	3	18	**100**
13	3	36	3	36	3	18	**100**
14	3	36	3	35	3	18	**99**
15	3	36	3	35	3	13	**90**
17	3	36	3	36	3	18	**100**
18	3	27	3	36	3	18	**92**
19	3	36	3	36	3	18	**100**
21	3	35	3	36	3	18	**99**
24	3	34	3	36	1	4	**-**
25	3	35	3	36	3	16	**95**
26	3	26	3	35	3	18	**90**
27	3	36	3	36	3	18	**100**
29	3	28	3	34	3	18	**91**
31	3	32	3	36	3	18	**96**
32	3	36	3	36	3	16	**96**
35	3	35	3	36	3	18	**99**
38	3	36	3	36	3	16	**96**
48	3	36	3	32	3	16	**93**
49	3	35	3	34	3	18	**97**
51	3	34	3	36	3	18	**98**
52	3	36	3	35	3	18	**99**
65	3	36	3	34	3	18	**98**
66	1	4	3	36	3	18	**-**
83	3	36	3	28	3	18	**93**
85	3	36	3	34	3	18	**98**
86	3	33	3	36	3	18	**97**
90	3	36	3	36	3	18	**100**
92	3	36	3	36	3	17	**98**
93	3	35	3	36	3	18	**99**
94	3	36	3	36	3	18	**100**
96	3	36	3	36	3	18	**100**
98	3	33	3	36	3	18	**97**

Lab Number	No of returns 2014	Total score 2014 (out of 36 for 3 returns)	Number of returns 2013	Total score 2013 (out of 36 for 3 returns)	Number of returns 2012	Total score 2012 (out of 18 for 3 returns)	Average score as a percentage over 3 years*.
101	3	31	3	35	3	18	**94**
102	3	35	3	36	3	18	**99**
104	3	34	3	28	3	16	**87**
106	3	31	3	36	3	18	**95**
108	3	31	3	35	3	18	**94**
111	3	36	3	36	3	18	**100**
113	3	34	3	30	3	10	**78**
114	3	33	3	32	3	14	**87**
119	3	31	3	36	3	18	**95**
120	3	26	3	36	3	18	**91**
121	3	35	3	36	3	18	**99**
126	3	34	3	36	3	18	**98**
128	3	35	3	36	3	18	**99**
130	3	36	3	36	3	18	**100**
132	3	36	3	34	3	18	**98**
133	2	24	3	36	3	18	**89**
134	3	31	1	8	0	n/a	**-**
135	3	36	3	36	3	18	**100**
137	3	35	3	36	3	18	**99**
138	1	8	3	32	3	18	**-**
139	3	24	3	36	3	16	**85**
141	3	34	3	32	3	18	**94**
142	0	0	3	36	3	13	**-**
143	3	28	3	36	3	18	**93**
144	3	35	3	35	3	18	**97**
146	3	35	3	36	3	18	**98**
147	3	36	3	36	-	-	**-**
148	3	30	3	36	3	18	**94**
149	3	34	2	24	3	18	**-**
151	3	30	n/a	n/a	2	3	**-**
152	3	33	2	22	3	13	**-**
153	3	34	3	36	3	18	**98**
154	3	35	n/a	n/a	n/a	n/a	**-**
155	3	36	3	36	3	18	**100**
156	3	36	3	32	3	18	**96**
157	3	22	3	35	3	18	**86**
158	3	23	3	33	-	-	**-**
159	3	32	3	36	3	18	**96**
160	3	36	3	28	3	16	**89**
163	3	21	3	36	3	18	**86**
164	3	36	3	36	2	12	**-**
165	3	30	3	36	3	18	**94**
166	3	36	3	30	3	11	**81**
167	1	12	3	36			**-**
168	3	36	3	34	3	14	**91**
170	3	34	3	30	3	16	**89**

Lab Number	No of returns 2014	Total score 2014 (out of 36 for 3 returns)	Number of returns 2013	Total score 2013 (out of 36 for 3 returns)	Number of returns 2012	Total score 2012 (out of 18 for 3 returns)	Average score as a percentage over 3 years*.
172	3	36	3	35	3	18	**99**
174	3	34	3	36	2	12	-
175	3	31	2	20			-
176	3	31	3	32			-
178	3	35	3	36	2	12	-
180	3	36	3	32	3	18	**96**
181	3	34	n/a	n/a	3	16	
182	3	32					-
183	3	35					-
184	3	35					-
185	3	34					-
186	3	31					-

* Average score only available of 3 years of full returns.

Your Laboratory OA Number in the above Table is

Commentary

This year's samples were perhaps more challenging that those in 2013 with only 36% of participants achieving maximum scores compared to 59% in 2012. In the first triplet of urine specimens, sample 215 proved slightly more challenging with a number of laboratories failing to identify the cyclic derivative of citrulline. Only 22 participants recognised this metabolite as significant and of those, 19 correctly associated it with citrullinaemia. The mass spectrum of the cyclic derivative of citrulline was given and we suggest this is added into your databases. Sample 214 was from patient with intermittent MSUD, the potential confusion for some was the relatively low abundance of pathognomonic metabolites (2-hydroxy-isovalerate and 2-hydroxy-3 methylvalerate) compared to that often observed in classical MSUD.

In the second distribution, sample 219 provided some challenges. This was mostly due to participants trying to find a diagnosis. Only 71/92 of participants correctly suggested that treatment with L-dopa was the most likely cause.

In the final set of three specimens provided the most 'unusual' sample sent was 221. This was from a patient with a biochemically and genetically confirmed glutaric aciduria type 3 (GA3). Biochemically it is difficult to distinguish between glutaric aciduria type 3 or a generalised mitochondrial dysfunction or riboflavin responsive glutaric aciduria type 2 on riboflavin and the scores provided reflected this difficulty. GA3 is considered a benign disorder, however, in the case of this patient who presented acutely unwell at the age of 24 years, following a brief vomiting illness, the concomitant GA3 pathology potentially contributed to her pathogenesis. She was admitted to the ITU requiring ventilation for cerebral agitation. This acute decompensation was responsive both clinically and biochemically to riboflavin administration. It is also noted that patient also has complicating medical conditions included beta-thalassemia, coeliac disease, hypothyroidism and osteoporosis.

This is an interesting case, which raises the possibility and discussion of whether this condition is truly a benign disorder. This case was presented at the SSIEM meeting in September 2014 (see attached poster) in addition to another poster also referring to a possible clinical phenotype of GA3. (Glutaric aciduria type 3: is there a distinctive phenotype? Kitzler T M., Feigenbaum A,

Siriwardena K, Spector E, Waters P J, Al-Hertani W. Mcgill University Health Centre, Montreal, Quebec Canada).

It is appreciated that in all the cases the urine organic acid profile is only part of the diagnostic profile and where the organic acid profile does not give a clear diagnosis the further investigations box is key when it comes to scoring. The 'Further investigations' box should indicate any additional investigations you consider necessary to interpret or confirm conclusions based on the analytical results. The 'Additional comments' box may also be used for caveats or to suggest other lines of investigation based on the clinical presentation rather than the analytical findings. Suggestions should follow a logical hierarchy with simple group investigations such as amino acid chromatography or blood-spot acylcarnitine profiling (if indicated) taking precedence over much more specific investigations such as gene sequencing.

Certificates of Participation and Performance

We are required to define "Participation" and "Satisfactory Performance" for the purpose of the ERNDIM Annual Certificate which covers all ERNDIM schemes. For this urinary organic acid scheme we have defined "Participation" as requiring at least two returns during the year. Defining "Satisfactory Performance" is more problematical as in some years there are more difficult samples than in others.

The criteria for satisfactory performance remain the same as in 2012: for three returns a score of 11/18 (61%), which equates to a score of 22/36 under the new scoring system, and for two returns a score of 15 or more. On this basis all but one participating laboratories were deemed satisfactory. Satisfactory Performance" criteria are always somewhat arbitrary and in practice even a single missed or wrong diagnosis can be highly damaging. Thus the reason(s) for failure to correctly report on any of the samples in the scheme should be investigated locally and appropriate remedial action taken. Starting with the 2014 schemes the concept of 'critical error' will be introduced to the assessment of the qualitative urinary organic acid scheme.

A critical error is an error that would be unacceptable to the majority of labs (>95%) and would have a serious adverse effect on patient management. The introduction of critical error is on the advice of the Genetic Services Quality Committee (GSQC) of the European Society of Human Genetics (ESHG), which wants to see harmonisation across all European genetic EQA providers. A confirmed critical error will mean automatic classification as a poor performer. The final scoring of all qualitative schemes will be discussed at the Spring meeting of the Scientific Advisory Board (SAB) and all proposed critical errors will need to be ratified by the SAB before being confirmed.

We thank Lynne Darwin for administering our participant database and dealing with the returns, and Jennifer Watkinson for preparing and dispatching the samples. We hope that you continue to find this scheme useful.

Yours sincerely,

Dr Jane Dalley	Professor J R Bonham	Mrs Camilla Scott
Deputy Scientific Advisor		Scientific Advisor

Scheme organisers