ERNDIM
Qualitative Organic acids Urine Heidelberg

ANNUAL REPORT 2018

Scientific Advisor	Website for reporting results	Administration office:
Dr Claus-Dieter Langhans Metabolic Center Heidelberg Im Neuenheimer Feld 669 69120 Heidelberg Germany e-mail: claus-dieter.langhans@med.uni-heidelberg.de	Dr. Xavier Albe CSCQ Swiss Center for Quality Control 2 chemin du Petit-Bel-Air CH-1225 Chêne-Bourg Switzerland e-mail : Xavier.Albe@hcuge.ch	ERNDIM Adminsitration Office Manchester Centre for Genomic Medicine 6th Floor, St Mary's Hospital, Oxford Road, Manchester M13 9WL, United Kingdom. e-mail: admin@erndim.org

25 February 2019
30 July 2019*

1. Introduction

The ERNDIM Qualitative Organic Acids in urine scheme offers urine samples obtained from confirmed patients with confirmed diagnoses to enable laboratories to gain or maintain experience to identify organic acid disorders. The scheme is organised by Dr Claus-Dieter Langhans (metabolic center Heidelberg) in conjunction with CSCQ, the Swiss organisation for quality assurance in medical laboratories.

As in previous years, samples were sent out to cover the spectrum of what is typically observed in the metabolic laboratory. A mix of clearly diagnostic profiles and some more challenging profiles were provided. As in previous years normal profiles were also sent out. The requirement to interpret a normal profile, as such, is as important as correctly identifying abnormal profiles. Correctly identifying a profile as normal can avoid unnecessary further investigation and distress to the patient and family.

2. Participants

In 2018 73 laboratories from many different countries participated in the QLOU Heidelberg scheme. 0 laboratories were educational participants in 2018 (0 in 2017). They take part in all aspects of the scheme and receive interim reports with scores, but performance is not indicated on the ERNDIM certificate of performance.

The numbers of participants continues to increase and as a result a third organising centre, Barcelona, was added to the QLOU scheme for 2018 and participants were split between the three organising centres. New applicants will distributed between the Barcelona, Sheffield and Heidelberg qualitative urinary organic acid schemes which are run separately. The three organising laboratories each participate in the other's scheme by rotation.

Table 1: Geographical distribution of participants

Country	*Number of laboratories*		*Country*	*Number of laboratories*
Austria	3		Lithuania	1
Bulgaria	1		Luxembourg	1
Canada	10		Netherlands	8
China	3		Serbia	1
Croatia	1		Slovakia	1
Czech Republic	2		Slovenia	1
Estonia	2		Spain	1
Germany	18		Sri Lanka	1
India	1		Switzerland	3
Japan	1		Turkey	12
Latvia	1			

Version Number (& Date)	Amendments
[1] version 2 (30th July 2019)	• Page 2: Addition of reference to donated sample used in the 2018 scheme.

3. Design of the scheme and logistics

As usual, the samples used in 2018 were authentic human urine samples, 7 from affected patients and 2 from healthy individuals. Samples were selected by the Scientific Advisor and tested for suitability in the Scientific Advisor's laboratory. In 2018 CSCQ dispatched the QLOU EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports. Existing QLOU, ACDB, DPT and Urine MPS scheme participants can log on to the CSCQ results submission website at: https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php

To be able to continue this scheme we need a steady supply of new patient samples. Several laboratories have donated samples to the Urine QLOU scheme in the past, for which they are gratefully acknowledged. If you have one or more samples available and are willing to donate these to the scheme, please contact us at admin@erndim.org.
Laboratories which donate samples that are used in the scheme are eligible for a 20% discount on their participation in the QLOU scheme in the following year.

Table 2. Samples included in the 2018 ERNDIM QLOU Heidelberg scheme.
One sample in the 2018 scheme was donated by Labor Berlin GmbH, Berlin.

Survey, reporting deadline	Sample no.	Sample type
18-05-OUS, 04 June 2018	QLOU-DH-2018-A	Hawkinsinuria
	QLOU-DH-2018-B	Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)
	QLOU-DH-2018-C	Mevalonate kinase deficiency (MKD)
18-07-OUS, 30 July 2018	QLOU-DH-2018-D	Mitochondrial short-chain enoyl-CoA hydratase deficiency (ECHS1D)
	QLOU-DH-2018-E	Cobalamin C deficiency
	QLOU-DH-2018-F	Normal control
18-09-OUS, 01 October 2018	QLOU-DH-2018-G	Maple syrup urine disease
	QLOU-DH-2018-H	Propionic aciduria
	QLOU-DH-2018-I	Normal control

The scheme format was kept identical to those of previous years. Samples were shipped by regular mail. Details regarding stability of samples are provided in the sample package.

Participants submitted results to the CSCQ website https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php. The due dates for submitting results in 2018 were 04 June 2018, 30 July 2018 and 01 October 2018. The website includes a section to specify methods. Method specification is required for correct evaluation of the quantitative results

In 2018 a total of 71 reports were received for survey 1 (samples QLOU-DH-2018-A to QLOU-DH-2018-C), 70 reports for survey 2 (samples QLOU-DH-2018-D to QLOU-DH-2018-F) and 69 reports for survey 3 (samples QLOU-DH-2018-G to QLOU-DH-2018-I). 68 labs submitted results for all three surveys. 2 participants did not submit any report, while no other participant submitted one of the three reports and 3 other participants submitted two of the three reports.

Evaluation of results was performed using Excel with the submitted results extracted from the database by the website manager.

4. Scoring of results

A scoring system was developed in 2012 and approved by the ERNDIM Scientific Advisory Board. Similar to other qualitative (proficiency testing) ERNDIM schemes, the maximum score for a sample is 4 points. Scores are allocated to different elements of the results reported (Table 3).
Qualitative results and diagnostic proficiency of the 2017 samples were scored using the criteria given in Table 4 and 5. These criteria have been set by the Scientific Advisor, approved by the Scientific Advisory Board. The final decision about scoring of the scheme is made in the Scientific Advisory Board (SAB) during the Autumn meeting (November 29, 2018 for the 2018 scheme).

A note on scoring of diagnostic proficiency and the use of check boxes and the comment box:

To indicate the most likely diagnosis check boxes must be used to facilitate evaluation of results. The use of the 'comments' box in the website form is recommended to explain your interpretation of results. Comments will be taken into account to score interpretation.

Table 3. General criteria used to score results

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

Starting with the 2014 schemes the concept of 'critical error' is introduced to the assessment of the qualitative schemes. Labs failing to make a correct diagnosis of a sample considered eligible for this category will be deemed not to have reached a satisfactory performance even if their total points for the year is sufficient according to the requirement set by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on November 29, 2018. Sample QLOU-DH-2018-B was eligible for critical error. Amongst the reports of regular participants seven critical errors were identified in 2018. Details are given under item 7 'Results of individual samples and evaluation of reporting'.

We are required to define "Participation" 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. Failure to meet this requirement will result in the certificate of participation showing 'non-submitter' rather than 'satisfactory' or 'unsatisfactory'.

In 2018 the satisfactory performance score has changed from 61% to 70% which equates to 25/36 for 3 returns and 17/24 for two returns.

5. Communication of results

Interim reports with diagnoses, summaries of the results submitted and interim scores were made available July 31, 2018 (survey 18-05-OUH), October 4, 2018 (survey 18-07-OUH) and December 7, 2018 (survey 18-09-OUH).

ERNDIM provides a single certificate for all its schemes with details of participation and performance.

Seven Performance Support letters will be sent for the 2018 surveys. None of these 7 participants have also received a performance support letter in 2017 or 2016. Unsatisfactory performance (either due to overall score or due to critical error) within an EQA scheme for at least 2 out of 3 years that the participant has subscribed for will result in a notification letter of unsatisfactory performance to the quality manager or head of department.
For the 2017 scheme no Performance Support letters were sent.

6. Proficiency of the 2018 surveys

In 2018, 68 participants submitted 3 reports including 0 educational participants. From the 73 ordinary (non-educational) participants 60 (82%) achieved satisfactory performance (score ≥ *25 / 17*, no critical error). 13 participants did not accomplish satisfactory performance, including 2 due to incomplete submission of results (i.e. no report or 1 survey report submitted instead of 2 reports).
Overall proficiencies of each sample are depicted in Table 6.

Table 4. Overall proficiencies of the 2018 surveys.

Sample ID	Sample type	Proficiency (%)
QLOU-DH-2018-A	Hawkinsinuria	76
QLOU-DH-2018-B	Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)	79
QLOU-DH-2018-C	Mevalonate kinase deficiency (MKD)	99
QLOU-DH-2018-D	Mitochondrial short-chain enoyl-CoA hydratase deficiency (ECHS1D)	77
QLOU-DH-2018-E	Cobalamin C deficiency	71
QLOU-DH-2018-F	Normal control	83
QLOU-DH-2018-G	Maple syrup urine disease	94
QLOU-DH-2018-H	Propionic aciduria	99
QLOU-DH-2018-I	Normal control	87

7. Results of individual samples and evaluation of reporting

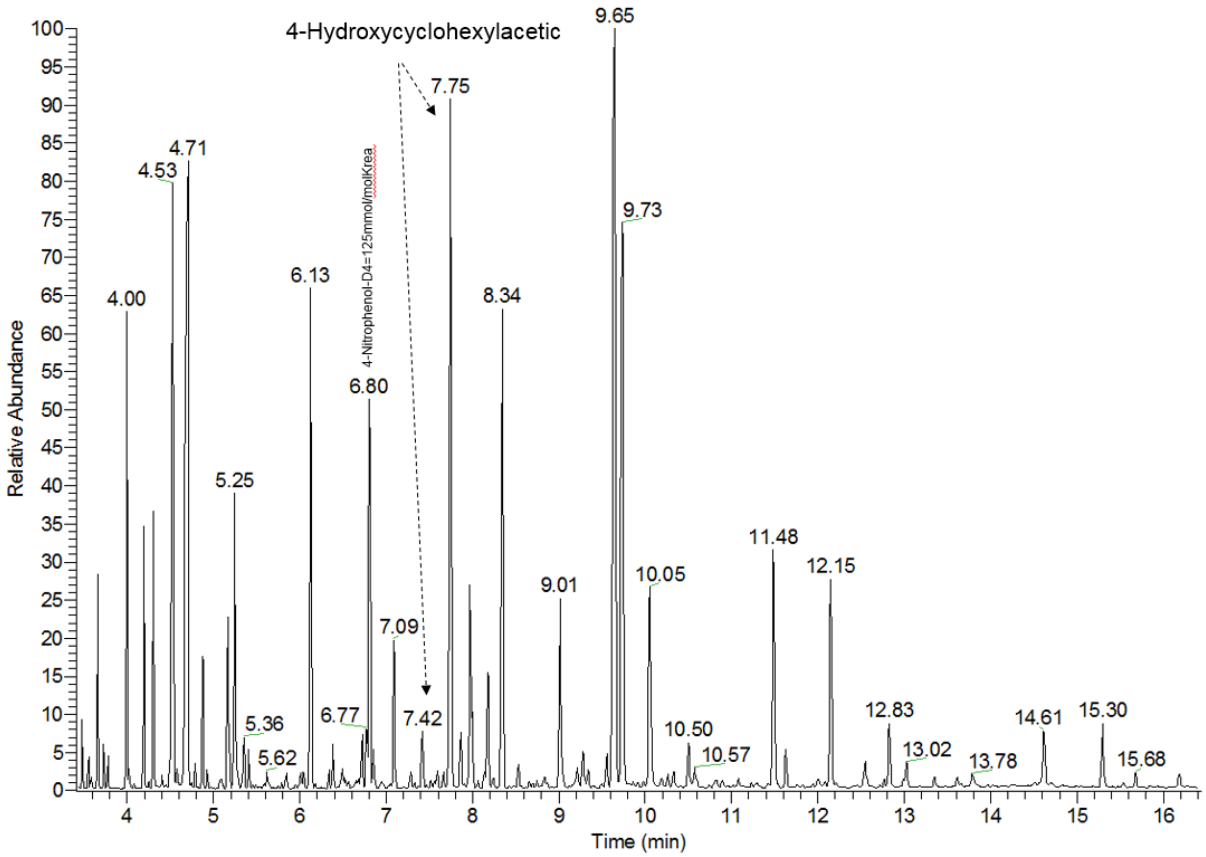
Sample QLOU-DH-2018-A:

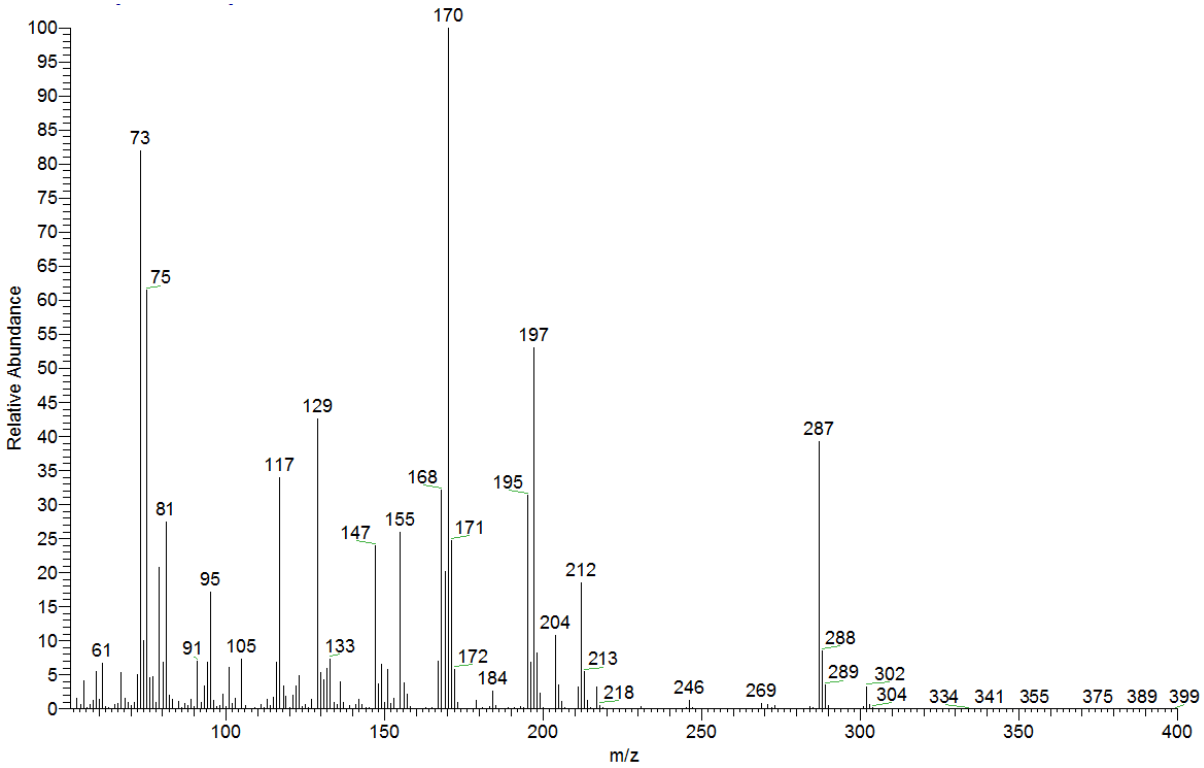
Patient details: 17-year-old girl, who presented at 18 months of age with failure to thrive, anemia and metabolic acidosis

Known diagnosis: Hawkinsinuria

Analytical details: The key finding was the detection of increased 4-hydroxycyclohexylacetic acid, described by 49/71 participants (70%). 4 participants reported hawkinsin.

Interpretation: The correct diagnosis of hawkinsinuria depends exclusively on the correct analytical finding of the key metabolite. The overall diagnostic performance was only 76%.





Mass spectrum of 4-hydroxycyclohexylacetic-2TMS

Sample QLOU-DH-2018-B:

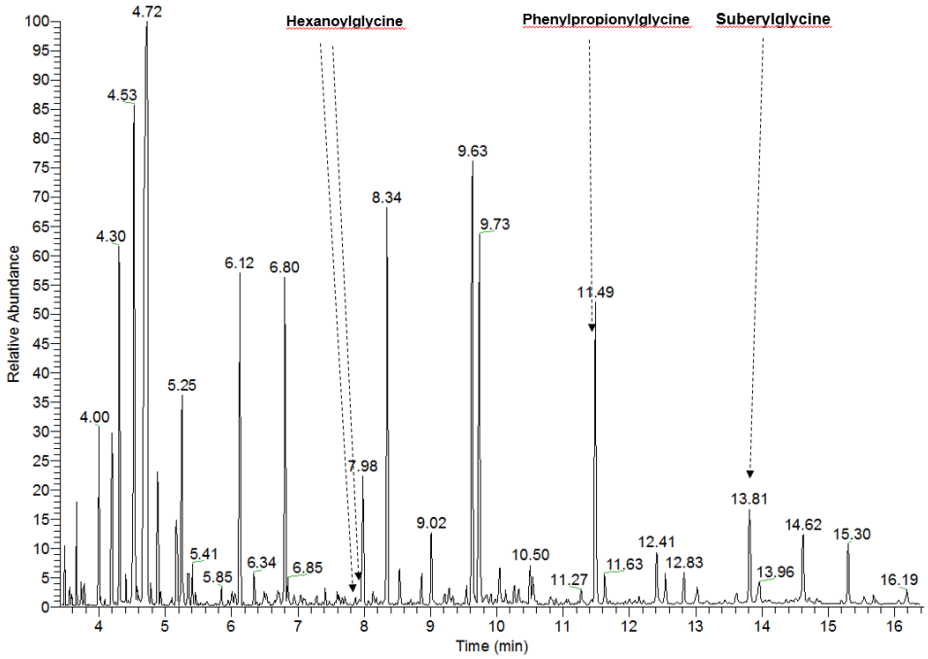
Patient details: 2-year-old boy with hypoglycemia during febrile illness

Known diagnosis: Medium chain acyl-CoA dehydrogenase deficiency (MCADD)

Analytical details: Clearly elevated amounts of hexanoylglycine, suberylglycine, and phenylpropionylglycine. These abnormalities were found by 55/71 participants (79%).
The detection of these glycine derivatives is vital for the correct diagnosis. Requirements for an effective analytical method are high, with the main focus on an optimal maintenance of the analytical equipment.

Interpretation: Diagnostic performance for MCADD was 79%. Thirteen participants considered the urine normal but six of them recommended further investigations that were suitable to come to the correct diagnosis (e.g. determination of acylcarnitines in DBS).

This sample was considered by the SAB to be eligible for critical error, as is the case with 7 participants who reported "normal" without further recommendations.



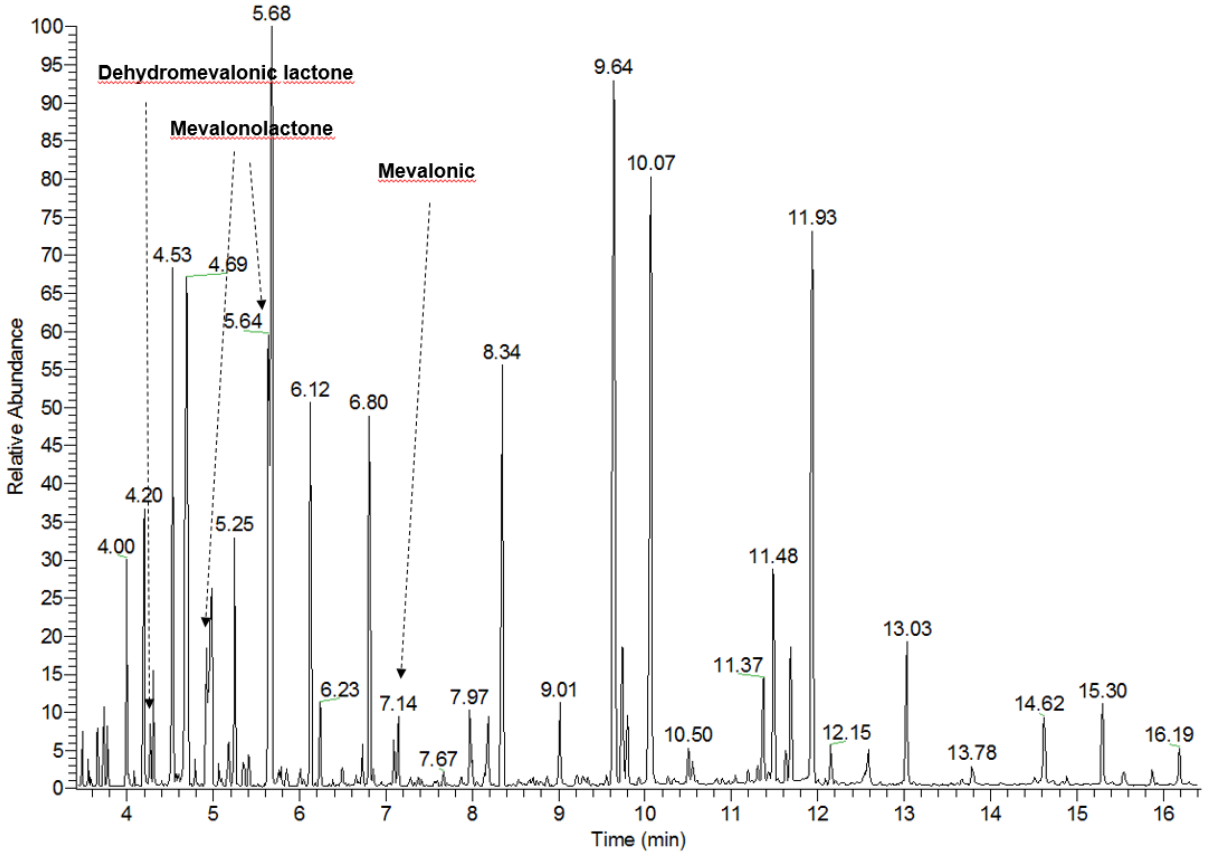
Sample QLOU-DH-2018-C:

Patient details: 11-year-old boy with global developmental delay, ataxia and hypotonia

Known diagnosis: Mevalonic aciduria (MVA)

Analytical details: Elevated amounts mevalonolactone and/or mevalonic acid acid were detected by all labs.

Interpretation: All participants diagnosed MVA. The overall diagnostic performance was 99%. One participant supposed the excreted mevalonic acid to originate from medication or diet but advised for molecular testing of the MVK gene. One point was subtracted for this diagnosis.



Sample QLOU-DH-2018-D:

Patient details: 2-year-old boy with developmental delay, admitted due to severe metabolic acidosis

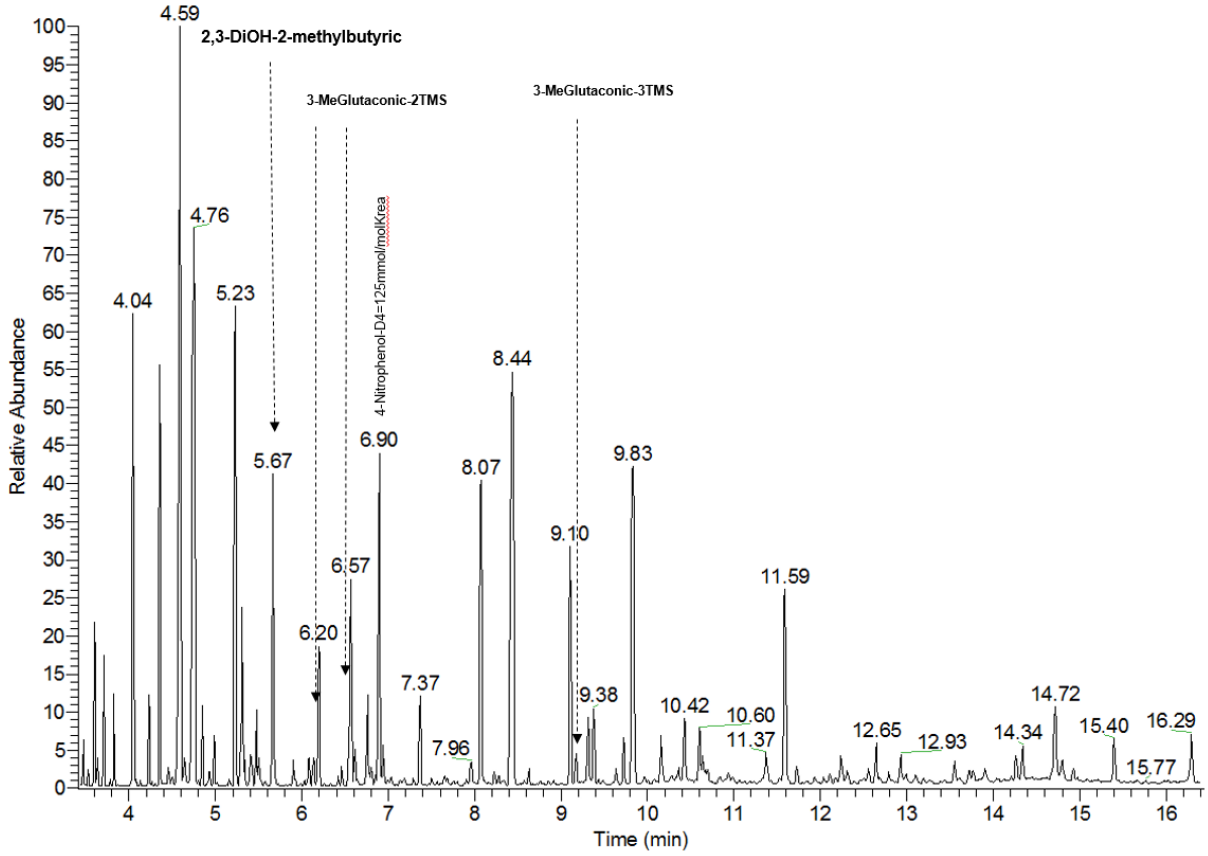
Known diagnosis: Short-chain enoyl-CoA hydratase deficiency (SCEH/ECHS1D)

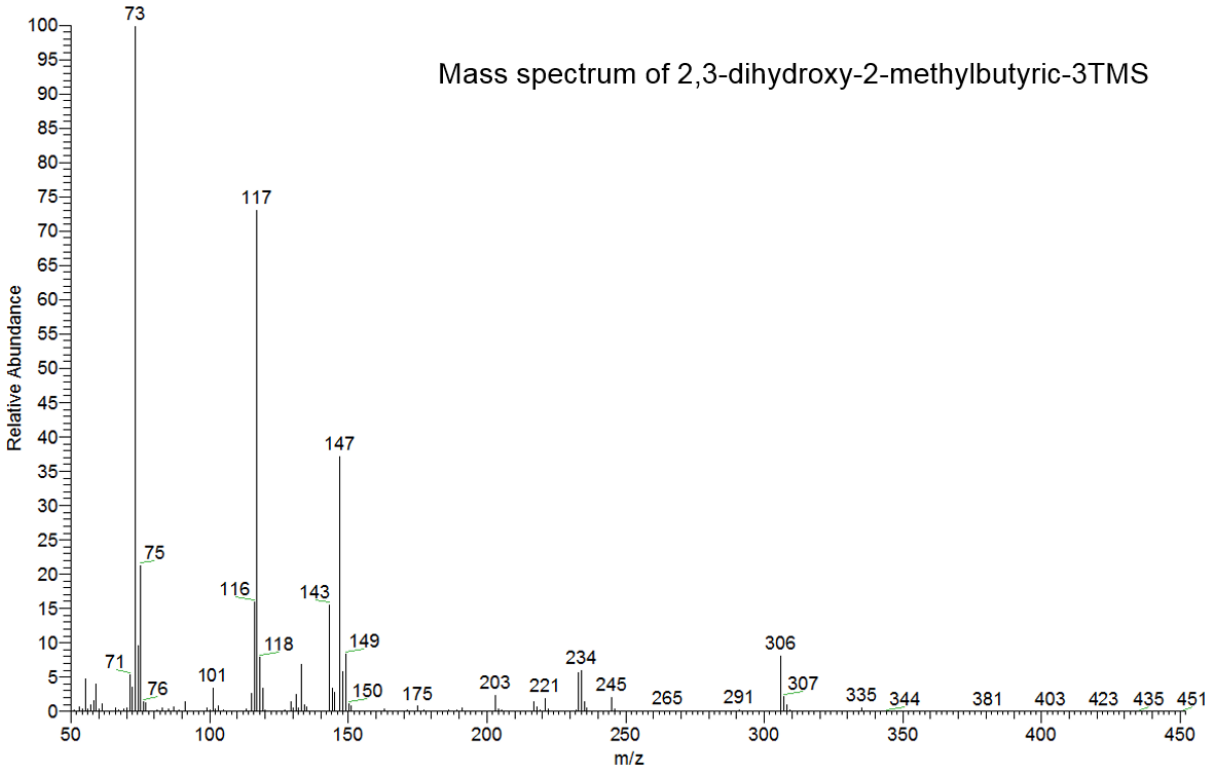
Analytical details: The key metabolite in this sample is 2,3-dihydroxy-2-methylbutyric acid which appears as a prominent peak in the chromatogram. The identification of this metabolite scored 2 points (30/70). One point was given for the detection of increased 3-methylglutaconic acid (36/70) or increased 3-hydroxyglutaric acid (13/70).

Interpretation: 30/70 participants diagnosed SCEH/ECHS1D or 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency. Both diagnoses are associated with the excretion of 2,3-dihydroxy-2-methylbutyric acid. This was scored 2 points. All other diagnoses related to 3-methylglutaconic acid and 3-hydroxyglutaric acid scored 1 point.
A normal urine was supposed by 17/70 participants.
The overall diagnostic performance was 76%.

A urine sample from a patient with ECHS1D was circulated for the first time in 2016.
Fifty-four participants have been registered for QLOU Heidelberg in both years (2016 and 2018). Six of them reported a normal diagnosis in both years (6/54, 11%) whereas five labs who gave a normal diagnosis in 2016 diagnosed ECHS1D correctly in 2018 (5/54, 9%). Twenty-three participants who reported other diagnoses like mitochondriopathy or 3-methylglutaconic acidurias in 2016 also diagnosed correctly ECHS1D in 2018 (23/54, 43%). Overall it seems that there was 52% improvement in performance for participants active in both years.

The SAB considered this sample not to be eligible for critical error, but scores should be taken into account in calculating overall performance.





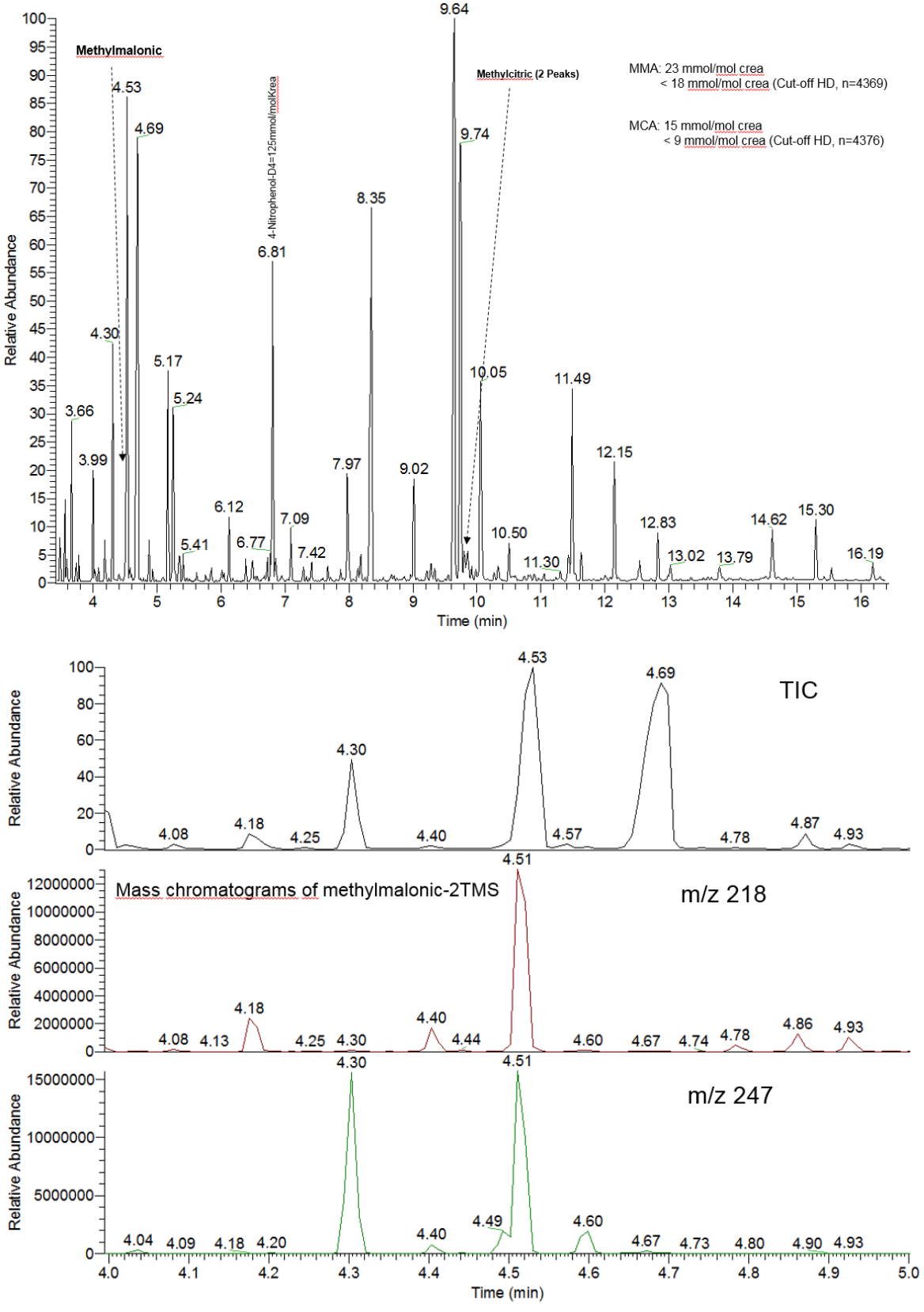
Sample QLOU-DH-2018-E:

Patient details:	6-year-old girl, who presented in infancy with growth retardation and hypotonia, currently on treatment
Known diagnosis:	cobalamine C (cbl C) deficiency

Analytical details: Methylmalonic acid and methylcitric acid are clearly elevated. However the excreted amounts are in the lower concentration range which makes the identification difficult. The identification of these metabolites scored 2 points (53/70 and 19/70).

Interpretation: In total 50/70 participants diagnosed methylmalonic aciduria or one of its subgroups. 16% of the participants pointed directly to cbl C deficiency (11/70).
24% of the participants reported a normal profile.
The overall diagnostic performance was 71%.

Even though missing methylmalonic acid and/or methylcitric acid could be harmful to patients, the SAB considered this sample not to be eligible for critical error, but scores should be taken into account in calculating overall performance.



Sample QLOU-DH-2018-F:

Patient details: 5-year-old boy, ataxia after infection

Known diagnosis: Normal sample, obtained from a healthy child
The sample was obtained from a healthy child.

Analytical details / Interpretation: The organic acid profile of this urine showed no abnormalities and this was reported by 83% of the labs.

Depending on the analytical findings some participants diagnosed 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (1), mitochondrial respiratory chain disorder (1), OTC deficiency (1), urea cycle defect (1), cerebellitis (1), defect in leucine degradative pathway possible (1) and mild elevation of tiglylglycine (1).
We subtracted one or two points for any given diagnosis other than normal, depending on the advice for further investigations.

The overall diagnostic performance was 83%.

Sample QLOU-DH-2018-G:

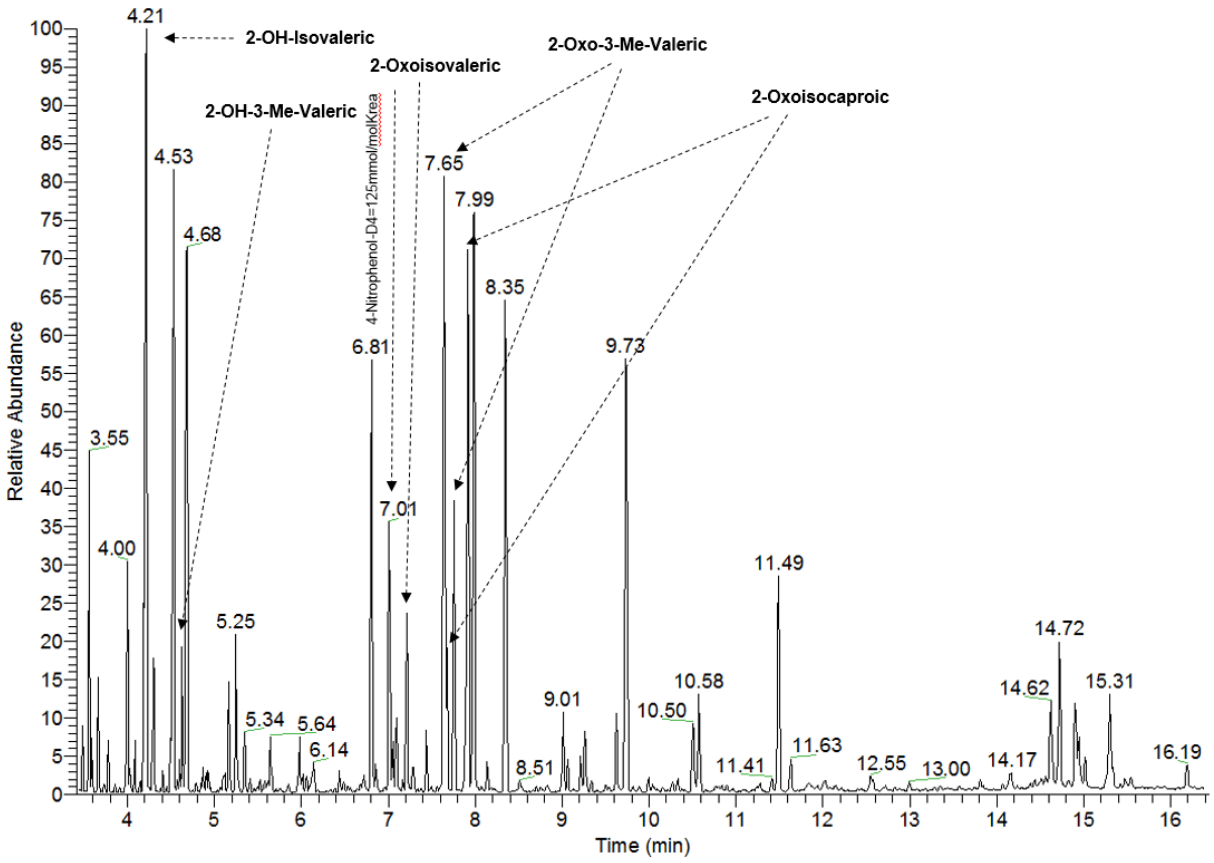
Patient details: 8-year-old boy with episodes of drowsiness and ataxia during febrile ill-ness

Known diagnosis: maple syrup urine disease (MSUD)

Analytical details: The chromatogram shows noticeable peaks of branched-chain hydroxyl- and oxoacids 2-hydroxyisovaleric acid, 2-hydroxy-3-methylvaleric acid, 2-oxoisovaleric acid and 2-oxo-3-methylvaleric acid. Identification of these metabolites is vital for the correct diagnosis and they were reported by nearly all participants (67/69).

Interpretation: 95% of the participants diagnosed MSUD. Two responders gave no diagnosis and scored zero points for interpretation.

The overall diagnostic performance was 83%.



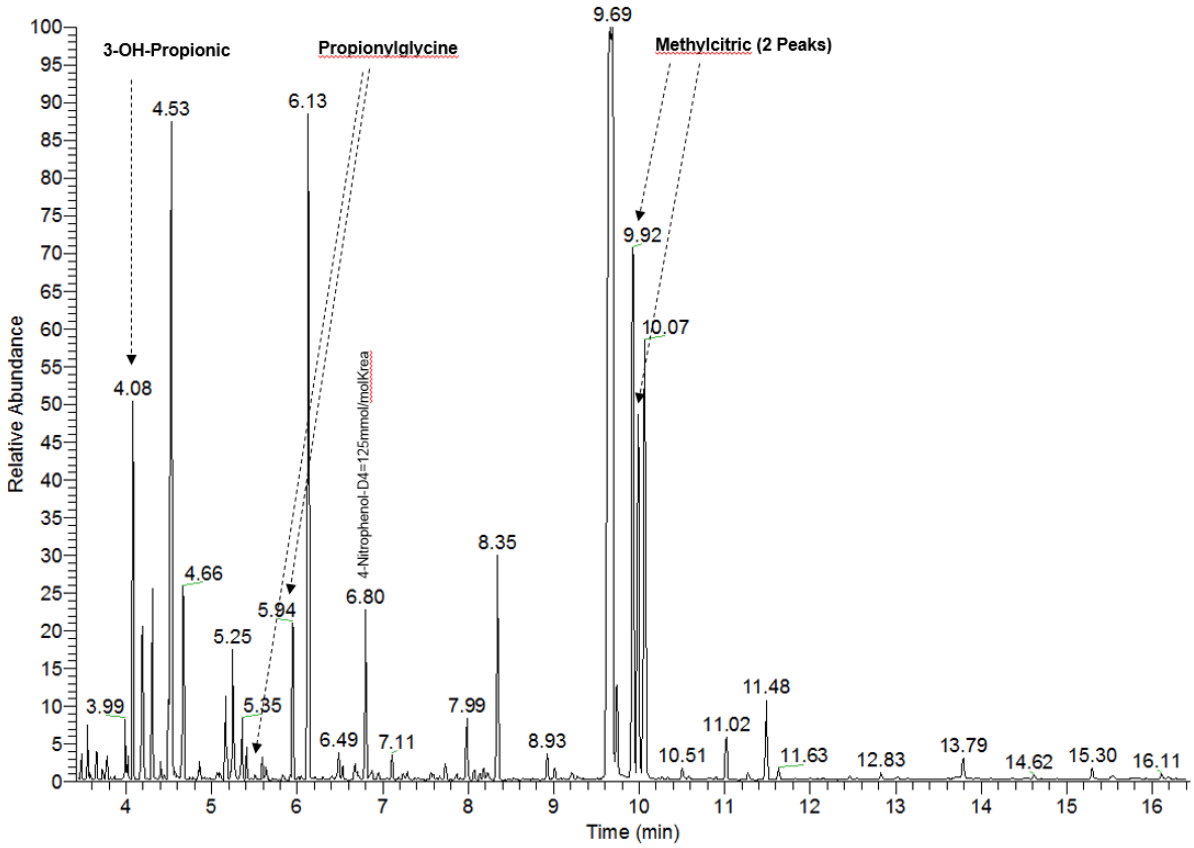
Sample QLOU-DH-2018-H:

Patient details: 13-year-old girl admitted with metabolic acidosis, hyperammonemia

Known diagnosis: Propionic aciduria, propionyl-CoA carboxylase deficiency, PCC deficiency

Analytical details: The relevant metabolites 3-hydroxypropionic acid, methylcitric acid, and propionylglycine were detected by all participants.

Interpretation: 66/69 participants diagnosed propionic aciduria and scored two points for interpretation. One point was given for biotinidase deficiency (1/69) and multiple carboxylase deficiency (1/69)
This was a straightforward sample with a very high overall proficiency of 99%



Sample QLOU-DH-2018-I:

Patient details: 6-year-old girl with speech problems and lack of concentration

Known diagnosis: Normal sample, obtained from a healthy child.
Analytical details: No abnormalities were reported by 34/69 participants (49%). 14 labs (20%) found several elevated metabolites, mostly mentioned was 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA).

Interpretation: In total 61/69 participants regarded this urine to be normal.
Depending on the analytical findings several diagnoses were suggested by eight responders.
In first place was autism (3/69) followed by 3-hydroxy-3-methylglutaryl CoA (HMGCoA) lyase deficiency (1/69), benzoate treatment (1/69), glutaric aciduria type 2 (1/69), mild ketosis (1/69), and urea cycle disorders (1/69).
One point was subtracted for any other diagnosis than normal.
The overall diagnostic performance was 87%.

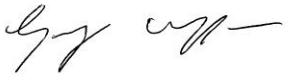
8. Preview of the scheme in 2019

The format of the QLOU 2019 scheme will be similar to that of previous years.

Changes planned for 2019:
Interim reports are intended to be produced automatically by a software developed by CSCQ. This is already working in the proficiency testing schemes and has to be adopted to the QLOU requirements.

25 February 2019





Dr. C. D. Langhans	**Dr. V. Peters**	**Prof. Dr. G. F. Hoffmann**
Scientific Advisor		*Director*
Laboratory of Metabolic Diseases	*Laboratory of Metabolic Diseases*	*Department of General Paediatrics*

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