ERNDIM
Acylcarnitines in DBS
Heidelberg

ANNUAL REPORT 2020

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1. Introduction

The ERNDIM Acylcarnitine in dried blood spots scheme offers dried blood spots obtained from confirmed patients with confirmed diagnoses to enable laboratories to gain or maintain experience to identify organoacidopathies and fatty acid β-oxidation defects. 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 2020 42 laboratories from many different countries participated in the ACDB Heidelberg scheme.

There was no educational participant in 2020 (one in 2019). Educational participants 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.

Participants and new applicants will distributed between the Heidelberg, London and Rome acylcarnitine in dried blood spots schemes which are run separately. The three organising laboratories each participate in the other's scheme by rotation.

Geographical distribution of participants				
Country	*Number of laboratories*		*Country*	*Number of laboratories*
Argentina	3		Luxembourg	1
Austria	1		Netherlands	5
China	2		Poland	1
Estonia	1		Turkey	1
France	16		UK	1
Germany	8		USA	1
Japan	1			

[1] If these scheme instructions are not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document

3. Design of the scheme and logistics

As usual, the samples used in 2020 were authentic human blood spot samples, 5 from affected patients and one from a healthy individual.
All samples selected by the Scientific Advisor are prepared from 30-50µl of lithium heparin anticoagulated whole blood on Whatman (Schleicher & Schuell) 903™ paper. All samples are obtained following local ethical and consent guidelines.
In 2020 CSCQ dispatched the ACDB 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
Labelled copies of scan/chromatograms can be uploaded on the CSCQ website.

4. Schedule of the scheme

Time schedule in the 2020 ERNDIM ACDB Heidelberg scheme.

	1st Submission Round	**2nd Submission Round**
Sample ID's:	ACDB-DH-2020-A ACDB-DH-2020-B ACDB-DH-2020-C	ACDB-DH-2020-D ACDB-DH-2020-E ACDB-DH-2020-F
Shipment of samples	February 12th, 2020	
Start of analysis (clinical data available)	March 9th, 2020	June 8th, 2020
Reminder for result submission	March 23th, 2020	July 15th, 2020
Results submission deadline:	March 30th, 2020	July 22nd, 2020
Interim reports available on CSCQ website	September 1st, 2020	September 17th, 2020

To be able to continue this scheme we need a steady supply of new patient samples. Several laboratories have donated samples to the ACDB 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 ACDB scheme in the following year.

Samples included in the 2020 ERNDIM ACDB Heidelberg scheme.

Survey	Sample no.	Diagnosis
20-07-ACH	ACDB-DH-2020-A	long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
	ACDB-DH-2020-B	normal
	ACDB-DH-2020-C	**Common sample**: long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
20-09-ACH	ACDB-DH-2020-D	3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency
	ACDB-DH-2020-E	glutaric aciduria type I
	ACDB-DH-2020-F	isovaleric acidaemia

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.

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

5. Results

Returned results in the 2020 ERNDIM ACDB Heidelberg scheme.

Submissions	Number of laboratories	%
2	40	95
1	-	-
0	2	5

6. Website reporting

The website reporting system is compulsory for all centers. Please read carefully the following advice:

- Results
  - Give quantitative data as much as possible.
  - Enter the key metabolites with the evaluation **in the tables** even if you don't give quantitative data.
  - If the profile is normal: enter "Normal profile" in "Key metabolites".
  - **Don't enter results in the "comments" window**, **otherwise your results will not be included in the evaluation program.**
- ***Diagnosis***
  - **Don't enter the diagnosis in the "comments" window**, **otherwise your results will not be included in the evaluation program.**
- Recommendations = **advice for further investigation**.
  - Scored together with the interpretative score.
  - Advice for treatment are not scored.
  - **Don't give advice for further investigation in "Comments on diagnosis"**: it will not be included in the evaluation program.

7. 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.
Qualitative results and diagnostic proficiency of the 2020 samples were scored using the criteria given below. 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 19th, 2020).

General criteria used to score results

Item	Description of scoring criteria	Score
Quantitative results	Correct classification of quantitative results (i.e. normal or increased) according to reference values	1
	Incorrect classification of quantitative results	0
Qualitative results	Correct results according to criteria set for the sample	1
	Incorrect: minimally required results not reported	0
Diagnostic proficiency	Correct according to criteria set for the sample	2
	Partially correct	1
	Unsatisfactory or misleading	0
	Maximum total score	**4**

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 19th, 2020.

Samples eligible for critical errors in the 2020 ERNDIM ACDB Heidelberg

Sample	Critical errors
ACDB-DH-2020-A	2
ACDB-DH-2020-C	2

Details are given under item 9 '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 acylcarnitine in dried blood spots scheme we have defined "**Participation**" as requiring **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'.

Satisfactory performance is defined as **70% of maximum score** which equates **17/24** points.

If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter.

8. Proficiency of the 2020 surveys

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

In 2020, 40 participants submitted 2 reports. From the 40 ordinary (non-educational) participants 38 (91%) achieved satisfactory performance (score ≥ *17*, no critical error). Four 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 the 2020 surveys.

Sample ID	Sample type	Proficiency (%)
ACDB-DH-2020-A	long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	88
ACDB-DH-2020-B	normal	83
ACDB-DH-2020-C	**Common sample**: long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	90
ACDB-DH-2020-D	3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency	93
ACDB-DH-2020-E	glutaric aciduria type I	100
ACDB-DH-2020-F	isovaleric acidaemia	100

Four Performance Support letters will be sent for the 2020 surveys. None of these participants have also received a performance support letter in 2019 or 2018. 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 2019 scheme six Performance Support letters were sent.

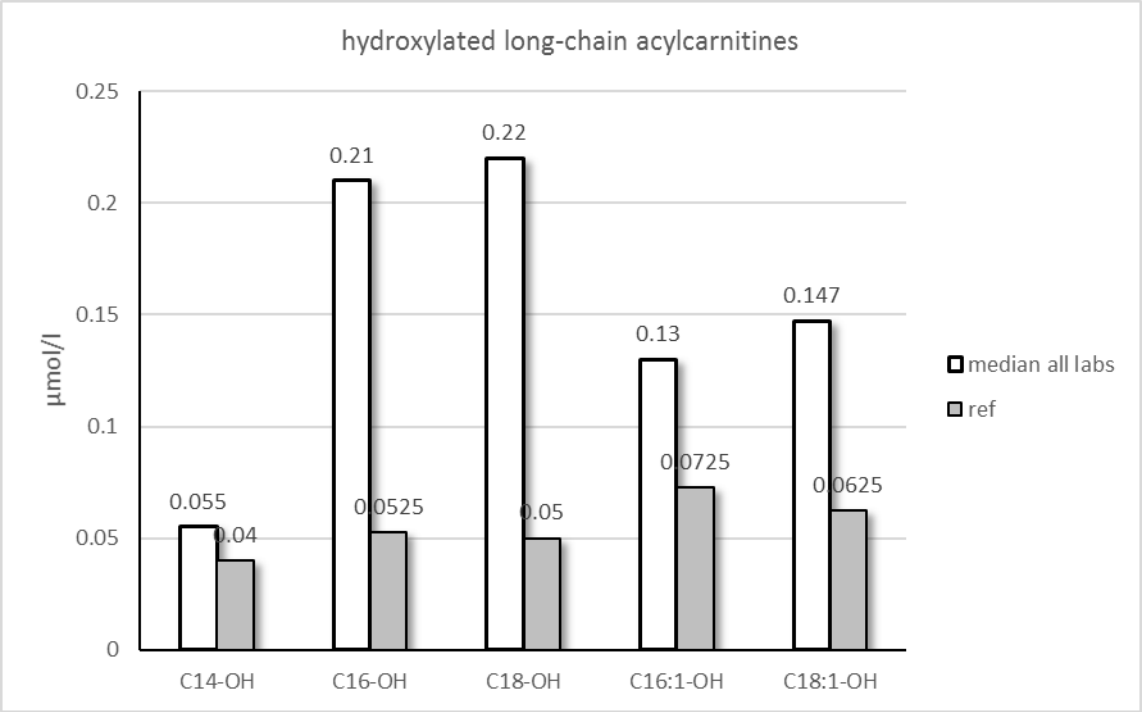
9. Results of individual samples and evaluation of reporting

Sample ACDB_DH_2020-A:

Patient details:	This child was referred to hospital with severe leg pain and dark urine
Known diagnosis:	long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

Analytical details: This sample showed elevated amounts of hydroxylated long-chain acylcarnitines, mainly C16-OH, C18:1-OH and C18-OH.

Overall 37/40 participants (93%) reported these metabolites.



Interpretation: 34/40 participants (85%) diagnosed long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) or mitochondrial trifunctional protein (MTP) deficiency.

Two participants (5%) supposed CPT 2 deficiency.

Critical error: In the November meeting the ERNDIM SAB considered the failure to detect elevated concentrations of hydroxylated long-chain acylcarnitines as a critical error. This was applied to two laboratories who gave a normal diagnosis.

Sample ACDB_DH_2020-B:

Patient details: 55-year old male presented with leg pain and weakness

Known diagnosis: normal control sample

Analytical details: This was a normal acylcarnitine profile.

Interpretation: 29/40 participants (73%) gave a normal diagnosis.

Other participants suggested vitamin B_{12} deficiency (4/40), CPT 2 deficiency (1/40), 3-hydroxy-3-methylglutaric aciduria (1/40), methylmalonyl-CoA mutase deficiency (1/40), multiple acyl-CoA dehydrogenase deficiency (1/40), treated MCAD deficiency (1/40), and VLCAD deficiency (1/40). One point was subtracted for any other diagnosis depending on the recommendations for further investigations.

Sample ACDB_DH_2020-C:

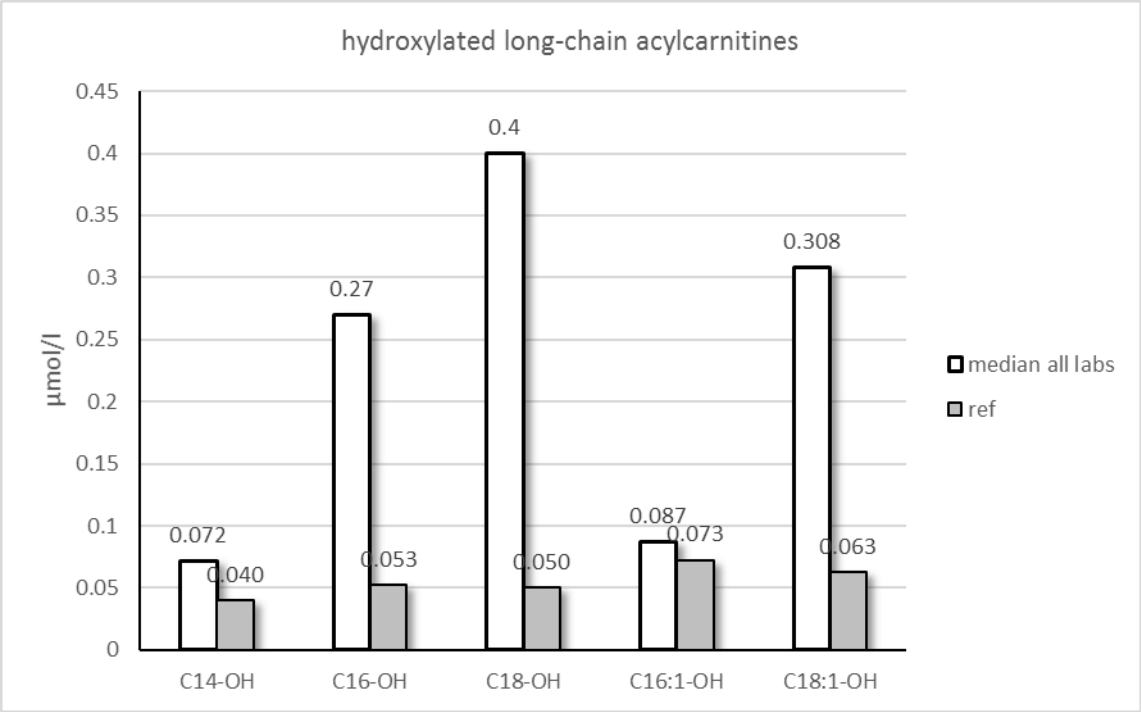
Patient details: Patient admitted at the age of 4 months for hypotonia, hypoglycemia, hypocalcemia and myoglobinuria. In treatment.

Known diagnosis: long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

Analytical details: This acylcarnitine profile also showed elevated amounts of hydroxylated long-chain acylcarnitines C16-OH, C16:1-OH, C18:1-OH, C18-OH.

35/40 laboratories reported these analytical findings. This outcome is comparable to sample #A.

Overall, the abnormalities were more pronounced in sample #C than in sample #A except for C16:1-OH-carnitine.



Interpretation: 36/40 participants (90%) diagnosed long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) or mitochondrial trifunctional protein (MTP) deficiency.

Two respondents suggested isovaleryl CoA dehydrogenase (IVD) deficiency and two others 3-HMG-CoA lyase deficiency.

Critical error: As in sample #A the ERNDIM SAB considered the failure to detect elevated concentrations of hydroxylated long-chain acylcarnitines as a critical error.

Two participants received critical errors also for this sample. They both gave a wrong diagnosis without suggesting suitable further examinations

This was a common sample distributed by the ACDB center Rome.

Organizing center	Participants (active/total)	Proficiency [%]
Heidelberg	40 / 42	90
London	42 / 44	95
Rome	45 / 41	89
Overall	123 / 131	**91**

Proficiency of all laboratories in diagnosing LCHAD deficiency was quite good with 91%.

The proficiency was similar between the three organizing centers with somewhat higher proficiency in the London scheme.

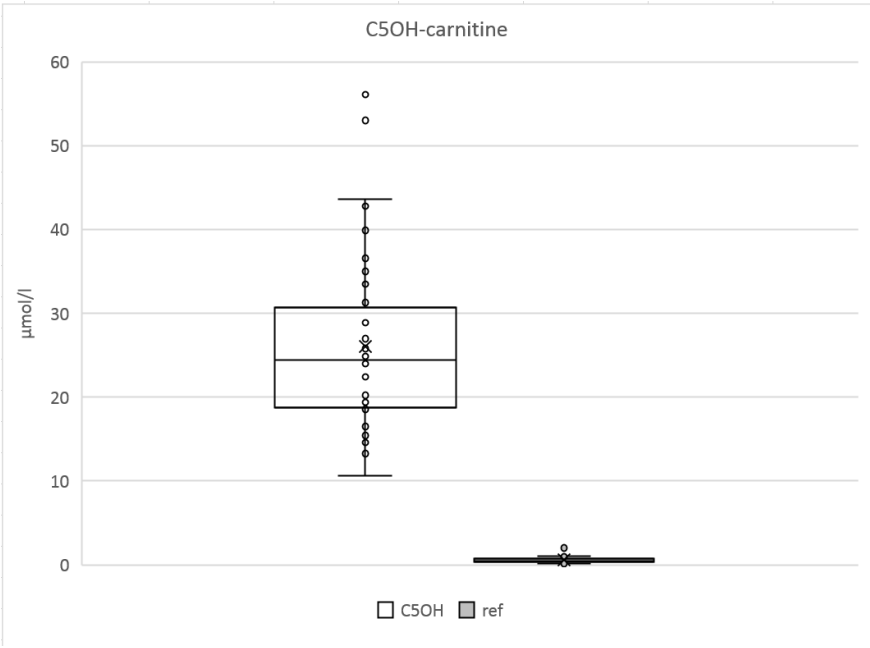
Sample ACDB_DH_2020-D:

Patient details: 4-year old boy evaluated for failure to thrive

Known diagnosis: 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency

Analytical details: Clearly elevated concentrations of C5-OH-carnitine. This was reported by all active participants (100%).

Analytical performance was 100%.



Interpretation: Most of the participants (33/40; 83%) diagnosed 3-MCC deficiency.

Some respondents suggested different diagnoses that also show elevated C5-OH-carnitine.
Three participants mentioned HMG-CoA lyase deficiency (8%) and two multiple carboxylase deficiency (5%).

Two laboratories diagnosed either 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency and mitochondrial acetoacetyl-CoA thiolase deficiency.

For scoring of the different diagnoses we considered the alternative diagnoses as well as the recommendations. One point was subtracted when 3-MCC deficiency was not mentioned or no advice for organic acid analysis was given.

The overall performance was 83%.

Sample ACDB_DH_2020-E:

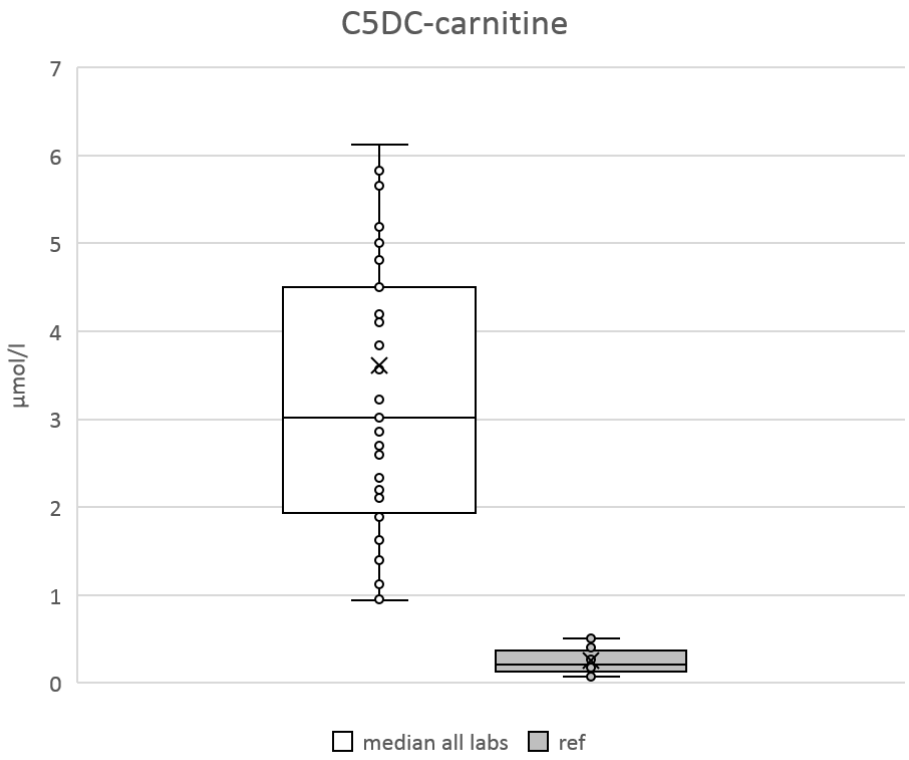
Patient details: 5-year old male, presented with loss of milestones after febrile infection

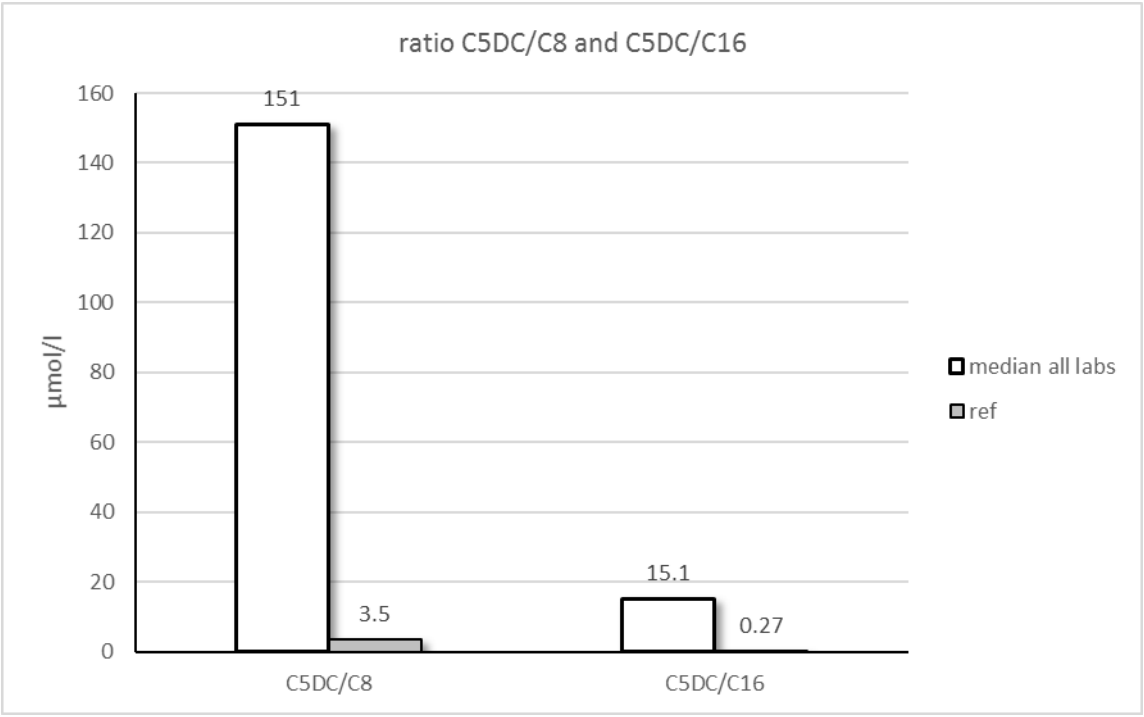
Known diagnosis: glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I)

Analytical details: the acylcarnitine profile showed elevated concentrations of C5DC-carnitine which was clearly identified by all participants.

Interpretation: All participants diagnosed glutaric aciduria type I

The overall performance was 100%.

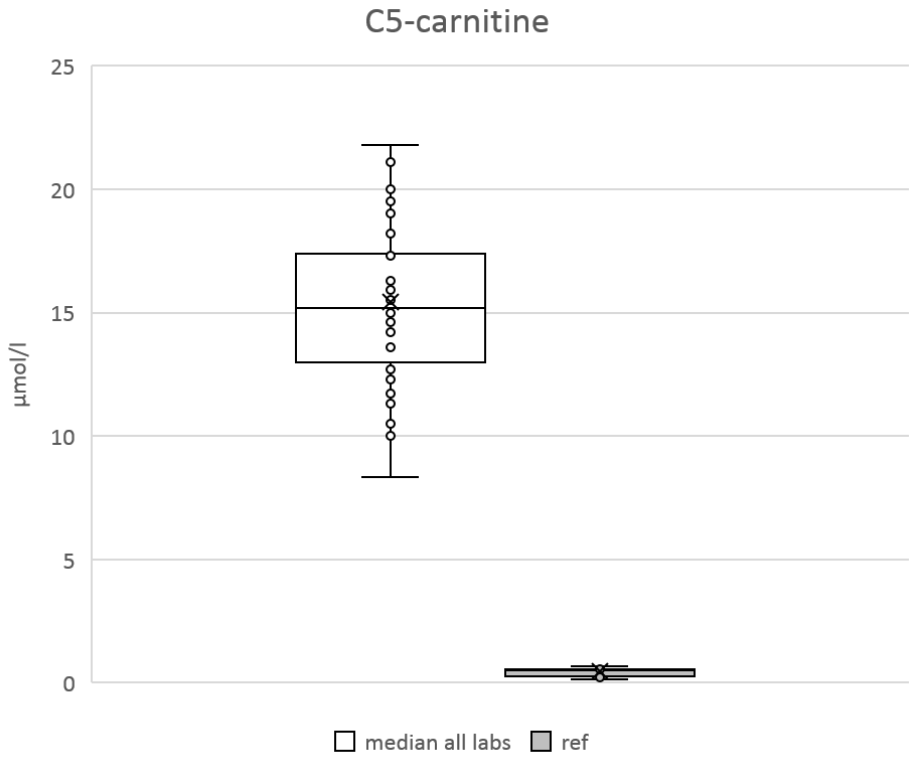




Sample ACDB_DH_2020-F:

Patient details:	8-year-old boy admitted with acute abdominal pain, ketoacidosis and abnormal breathing
Known diagnosis:	isovaleryl-CoA dehydrogenase deficiency (isovaleric acidaemia)
Analytical details:	Elevated C5-carnitine was the relevant feature in this sample.

Identification of this acylcarnitine was not a problem for all participants.



Interpretation: All participants diagnosed isovaleric acidaemia

The overall performance was 100%.

10. Scores of participants

The table presents detailed scores and performance data for all participants.

Scores and performance data were confirmed by the Scientific Advisory Board meeting in November 2020.

The anonymous data are accessible to all participants. Individual data are only visible to your laboratory

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
1	4	4	4	12	4	4	4	12	24	
2	4	4	4	12	4	4	4	12	24	
3	4	4	4	12	4	4	4	12	24	
4	4	4	4	12	4	4	4	12	24	
5	4	3	4	11	4	4	4	12	23	
6	4	4	4	12	4	4	4	12	24	
7	4	4	4	12	4	4	4	12	24	
8										non-submitter
9	4	3	4	11	4	4	4	12	23	
10	4	4	4	12	4	4	4	12	24	
11	4	4	4	12	4	4	4	12	24	
12	0	3	2	5	4	4	4	12	17	CE

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
13	4	3	4	11	3	4	4	11	22	
14	4	4	4	12	4	4	4	12	24	
15	4	4	4	12	4	4	4	12	24	
16	4	4	4	12	4	4	4	12	24	
17	4	4	4	12	4	4	4	12	24	
18	2	4	4	10	4	4	4	12	22	
19	4	4	4	12	4	4	4	12	24	
20	0	3	2	5	4	4	4	12	17	CE
21	4	4	4	12	4	4	4	12	24	
22	4	4	4	12	4	4	4	12	24	
23	4	4	4	12	4	4	4	12	24	
24	4	4	4	12	4	4	4	12	24	
25	4	4	4	12	4	4	4	12	24	
26	4	4	4	12	4	4	4	12	24	
27	3	3	2	8	4	4	4	12	20	
28	4	4	4	12	4	4	4	12	24	
29	4	4	4	12	4	4	4	12	24	
30	4	4	4	12	4	4	4	12	24	
31	4	4	4	12	3	4	4	11	23	
32	4	4	4	12	4	4	4	12	24	
33	4	4	4	12	4	4	4	12	24	
34	1	4	2	7	3	4	4	11	18	
35	4	4	4	12	4	4	4	12	24	
36	4	4	4	12	4	4	4	12	24	
37	4	4	4	12	4	4	4	12	24	
38	4	3	4	11	4	4	4	12	23	
39	4	4	4	12	4	4	4	12	24	
40	4	4	4	12	4	4	4	12	24	
41	4	4	4	12	4	4	4	12	24	
42										non-submitter

*) Educational sample
CE: Critical error
PP: Poor performance (on score)

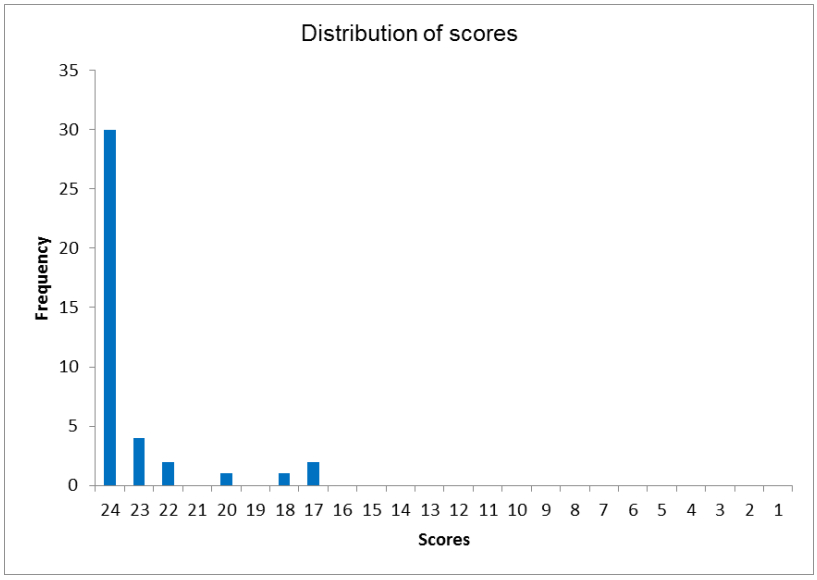


Fig: Distribution of scores on two submissions only

11. Preview of the scheme in 2021

The format of the ACDB 2021 scheme will be similar to that of previous years.

Changes planned for 2021:
Interim reports are intended to be produced automatically by a software developed by CSCQ.

January 11, 2021

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Please note:
This annual report is intended for participants of the ERNDIM ACDB scheme. The contents should not be used for any publication without permission of the scheme advisor

APPENDIX 1. **Change log (changes since the last version)**

Version Number	Published	Amendments
1	17 February 2021	2020 annual report published