ERNDIM Acylcarnitines in DBS London

ANNUAL REPORT 2020

Scientific Advisor	Website for reporting results	Administration office:
Charles Turner The WellChild Laboratory Arctic (1st Floor) Evelina London Children's Hospital St Thomas' Hospital London SE1 7EH, UK Chas.turner@kcl.ac.uk	Anthony Barrozo, João Diogo Amaral Rodrigues CSCQ Swiss Center for Quality Control 2 chemin du Petit-Bel-Air CH-1225 Chêne-Bourg Switzerland e-mail: Anthony.Barrozo@hcuge.ch JoaoDiogo.AmaralRodrigues@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

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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 organic acidopathies and fatty acid β-oxidation defects. The scheme is organised by Charles Turner, Evelina London Children's Hospital, 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 can also be 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 44 laboratories from many different countries participated in the ACDB London scheme. No laboratories were educational participants in 2020 (0 in 2019). 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.

Participants and new applicants will distributed between the Heidelberg, London and Rom acylcarnitine in dried blood spots 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*
Australia	4		Qatar	1
Brazil	1		Russia	1
Canada	4		Sultanate of Oman	1
Chile	1		Taiwan	1
Ireland	1		Turkey	2
Italy	13		UK	13
New Zealand	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, 6 from affected patients and 0 from healthy individuals.
All samples selected by the Scientific Advisor are prepared from 30-50µl of lithium heparin anticoagulated whole blood on PE226 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

Table 2: Time schedule in the 2020 ERNDIM ACDB London scheme. The closing date for the 1st submission round was extended to take account of problems associated with the Covid-19 pandemic.

	1st Submission Round	**2nd Submission Round**
Sample ID's:	ACDB-UL-2020-A ACDB-UL-2020-B ACDB-UL-2020-C	ACDB-UL-2020-D ACDB-UL-2020-E ACDB-UL-2020-F
Shipment of samples	February 11th, 2020	
Start of analysis (clinical data available)	Mar 9th, 2020	June 8th, 2020
Reminder for result submission	June 22nd, 2020	June 22nd, 2020
Results submission deadline:	June 29th, 2020	June 29th, 2020
Interim reports available on CSCQ website	Oct 14th 2020	Nov 11th 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.

Table 3: Samples included in the 2020 ERNDIM ACDB London scheme.

Survey	Sample no.	Diagnosis
20-03-ACL	ACDB-UL-2020-A	Carnitine acylcarnitine translocase deficiency (CACT: OMIM 212138)
	ACDB-UL-2020-B	Propionyl CoA carboxylase deficiency (PA: OMIM 606054)
	ACDB-UL-2020-C	Long chain hydroxyacyl CoA dehydrogenase deficiency (LCHADD: OMIM 609016)
20-06-ACL	ACDB-UL-2020-D	Medium chain acyl CoA dehydrogenase deficiency (MCADD: OMIM 201450)
	ACDB-UL-2020-E	Mild variant of cobalamin C deficiency (CblC OMIM 277400)
	ACDB-UL-2020-F	Very long chain acyl CoA dehydrogenase deficiency (VLCADD, OMIM 201475)

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

Table 4: Receipt of results in the 2020 ERNDIM ACDB London scheme.

Survey	In time	Late	Total
20-03-ACL	42	0	42
20-06-ACL	41	0	41

Table 5: Returned results in the 2020 ERNDIM ACDB London scheme.

Submissions	Number of laboratories	%
2	41	93.3
1	1	2.2
0	2	4.5

6. Website reporting

The website reporting system is compulsory for all centres. 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 in Table 6. 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 21st, 2020).

Table 6: 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 (Table 4)	1

	Incorrect: minimally required results not reported	0
Diagnostic proficiency	Correct according to criteria set for the sample (Table 5)	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 21st, 2020.

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.

Table 7: Samples eligible for critical errors in the 2020 ERNDIM ACDB London

Sample	Critical errors
ACDB-UL-2020-B	0
ACDB-UL-2020-C	2
ACDB-UL-2020-F	0

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 to **14/20** points.

8. Proficiency of the 2020 surveys

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

In 2020, 41 participants submitted 2 reports including 0 educational participants. From the 44 ordinary (non-educational) participants 39 (89%) achieved satisfactory performance (score ≥ 14, no critical error). 5 participants did not accomplish satisfactory performance, including 3 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 8.

Table 8: Overall proficiencies of the 2020 surveys.

Sample ID	Sample type	Proficiency (%)
ACDB-UL-2020-A	Carnitine acylcarnitine translocase deficiency	74.4
ACDB-UL-2020-B	Propionyl CoA carboxylase deficiency	97.6
ACDB-UL-2020-C	Long chain hydroxyacyl CoA dehydrogenase deficiency	95.2
ACDB-UL-2020-D	Medium chain acyl CoA dehydrogenase deficiency	97.0
ACDB-UL-2020-E	Mild variant of cobalamin C deficiency	Educational
ACDB-UL-2020--F	Very long chain acyl CoA dehydrogenase deficiency	97.6

5 Performance Support letters will be sent for the 2020 surveys. 2 of these 5 participants also received a performance support letter in 2019, both for non-submission of results. 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 7 Performance Support letters were sent.

9. Results of individual samples and evaluation of reporting

ACDB-UL-2020-A. All respondents recognised a fatty acid oxidation defect in this sample, but full interpretation of the profile proved difficult. Six respondents suggested MCADD on the basis of the elevated C6, C8 and C8/C10 ratio, disregarding the long chain acylcarnitine abnormalities and the absence of an elevated C10:1. Nearly half suggested MADD, understandably, given the variability of acylcarnitine profile in that disorder, however the long-chain abnormalities in this profile would be very unusual. CACT deficiency, the true diagnosis, is another disorder where acylcarnitine profiles can vary dramatically, both within a patient depending on clinical situation, and between patients. There was relatively good proficiency on this sample and it was included in the scoring for 2020 following discussions in the ERNDIM Scientific Advisory Board.

ACDB-UL-2020-B. All respondents correctly reported significantly raised propionyl (C3) carnitine in this sample, and most suggested appropriate tests to clarify and confirm the location of the deficiency in the propionate pathway. Failure to recognise the raised C3 in this sample would have constituted a Critical Error.

ACDB-UL-2020-C. The overwhelming majority (40/42) of respondents detected the elevated long chain hydroxyacyl carnitines in this sample and suggested appropriate tests to clarify and confirm the diagnosis of LCHADD. This sample was common to all three sections of the ERNDIM Acylcarnitines in Dried Blood Scheme, supplied by Dr Cristiano Rizzo, Scientific advisor in Rome. Further clinical details were supplied as follows: “Patient admitted at the age of 4 months for hypotonia, hypoglycemia, hypocalcemia, myoglobinuria and cholestasis. Laboratory analyses showed elevated long-chain hydroxylated acylcarnitines in DBS and hydroxylated dicarboxylic organic acids in urine. Mutation analysis showed a homozygous 1528G-C mutation in the gene encoding long-chain hydroxyacyl-CoA dehydrogenase (HADHA; 600890). The mother developed HELLP syndrome during pregnancy. The communal sample C was taken at the age of 15, the patient showed peripheral neuropathy and retinopathy and was being treated with Vitamin D3, Vitamin B1 and Coenzyme Q10”.

Proficiency in the Rome section was 89%, and in the Heidelberg section was 90%. Combining results across the three ACDB centres gave a total of 122 laboratories who submitted results of which 112 were satisfactory, an overall proficiency of 92%.

There was a small elevation of isovaleryl (C5) carnitine in this sample and most of the unsatisfactory performers commented on the C5 but failed to detect any of the elevated long chain hydroxyacylcarnitine species. It was agreed, following discussions with the ERNDIM Scientific Advisory Board, that failure to detect these would constitute a Critical Error

ACDB-UL-2020-D. All respondents recognised the characteristic pattern for MCADD in this sample, with increases in C8, C6 and C10:1, and most suggested appropriate follow-up to confirm the diagnosis.

ACDB-UL-2020-E. The overwhelming majority of respondents considered this profile normal. Only 2 detected the mild but unequivocal elevation in methylmalonyl carnitine. The absence of a raised propionyl carnitine may mean that there was less scrutiny of the C4DC signal, or some laboratories using SRM/MRM acquisition may not include C4DC in their panel. Confusion with other isobaric compounds may also occur, particularly using an underivatised method, which may require alternative transitions or chromatography to resolve. The clinical presentation was not typical of the disorder, and therefore the clinical details given did not raise suspicion. It is nonetheless surprising that so many laboratories considered the profile entirely normal. This sample epitomises the difficulty of interpretation when patients are well

and on treatment. This sample was designated an educational sample and removed from the scoring following discussions at the ERNDIM Scientific Advisory Board.
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ACDB-UL-2020-F. All (41/41) respondents detected the elevated C14:1 and related acylcarnitines in this sample and most suggested appropriate tests to clarify and confirm the diagnosis of VLCADD.

10. Scores of participants

Table 9 presents detailed scores and performance data for all participants.

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

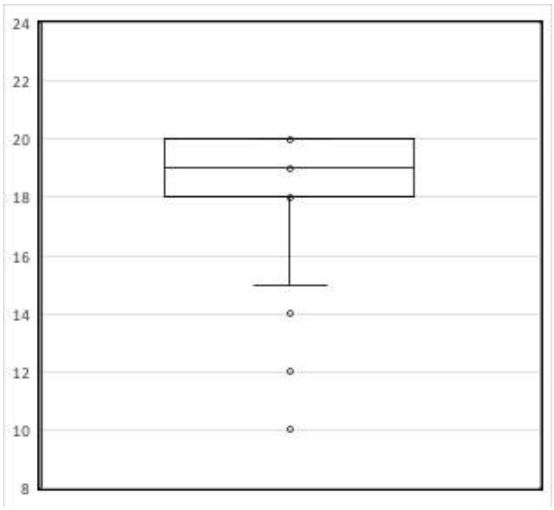
The anonymous data are accessible to all participants. Individual data are only visible to your laboratory.

Lab no	A	B	C	sum	D	E*	F	sum	Total score	Performance
1	4	4	4	12	4		4	8	20	
2	2	4	4	10	4		4	8	18	
3	4	2	4	10	4		4	8	18	
4	2	4	4	10					10	PS
5	4	4	4	12	4		4	8	20	
6	2	4	4	10	4		4	8	18	
7	3	4	4	11	4		4	8	19	
8	4	4	4	12	4		4	8	20	
9	4	4	4	12	4		4	8	20	
10	3	4	4	11	4		4	8	19	
11	3	4	4	11	4		4	8	19	
12										NS
13	3	4	4	11	4		4	8	19	
14	4	4	4	12	4		4	8	20	
15	3	4	4	11	4		4	8	19	
16	3	4	4	11	4		4	8	19	
17	4	4	4	12	4		4	8	20	
18	3	4	4	11	4		4	8	19	
19	3	4	4	11	4		4	8	19	
20	2	4	4	10	4		4	8	18	
21	3	4	4	11	4		4	8	19	
22	2	4	4	10	4		4	8	18	
23	4	4	0	8	3		3	6	14	CE
24	3	4	4	11	4		4	8	19	
25	4	4	4	12	4		4	8	20	
26	4	4	4	12	4		4	8	20	
27	2	4	4	10	4		4	8	18	
28										NS
29	3	4	4	11	4		4	8	19	
30	2	4	4	10	4		4	8	18	
31	4	4	4	12	4		4	8	20	

32	1	4	4	9	3		3	6	15	
33	1	2	4	7	3		4	7	14	
34	2	4	4	10	4		4	8	18	
35	4	4	4	12	4		4	8	20	
36	2	4	4	10	4		4	8	18	
37	2	4	4	10	4		4	8	18	
38	3	4	4	11	4		4	8	19	
39	4	4	4	12	4		4	8	20	
40	3	4	4	11	4		4	8	19	
41	2	4	4	10	4		4	8	18	
42	4	4	4	12	4		4	8	20	
43	2	4	4	10	4		4	8	18	
44	4	4	0	8	2		2	4	12	PP/CE

*) Educational sample
CE: Critical error
PP: Poor performance (on score)
PS: Partial submitter
NS: Non submitter

Figure 1: Boxplot presentation of all scores
Outliers result from contributing less than two submissions or low score



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. This is already working in the proficiency testing schemes and has to be adopted to the ACDB requirements.

Charles Turner
8Th March 2020
Scientific Advisor

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

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