ERNDIM Acylcarnitines in DBS London

ANNUAL REPORT 2019

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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 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 2019 44 laboratories from many different countries participated in the ACDB London scheme. No laboratories were educational participants in 2019 (0 in 2018). 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		New Zealand	1
Brazil	1		Poland	1
Canada	4		Qatar	1
Chile	1		Russia	1
Estonia	1		Sultanate of Oman	1
Germany	1		Taiwan	1
Ireland	1		Turkey	2
Italy	10		UK	13

3. Design of the scheme and logistics

As usual, the samples used in 2019 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 2019 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 2019 ERNDIM ACDB London scheme.

	1st Submission Round	2nd Submission Round
Sample ID's:	ACDB-UL-2019-A ACDB-UL-2019-B ACDB-UL-2019-C	ACDB-UL-2019-D ACDB-UL-2019-E ACDB-UL-2019-F
Shipment of samples	February 5th, 2019	
Start of analysis (clinical data available)	May 6th, 2019	July 1st, 2019
Reminder for result submission	May 20th, 2019	July 15th, 2019
Results submission deadline:	May 27th, 2019	July 22nd, 2019
Interim reports available on CSCQ website	Oct 15th 2019	Mar 6th 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 2019 ERNDIM ACDB London scheme.

Survey	Sample no.	Diagnosis
19-07-ACH	ACDB-UL-2019-A	Isovaleryl CoA dehydrogenase deficiency OMIM 243500
	ACDB-UL-2019-B	methylmalonyl CoA mutase deficiency, OMIM 251000
	ACDB-UL-2019-C	carnitine palmitoyl transferase type 2 OMIM 255110
19-09-ACH	ACDB-UL-2019-D	propionic acidaemia OMIM 6060540
	ACDB-UL-2019-E	multiple acyl CoA dehydrogenase deficiency OMIM 231680
	ACDB-UL-2019-F	mild multiple acyl CoA dehydrogenase deficiency OMIM 231680

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 2019 ERNDIM ACDB London scheme.

Survey	In time	Late	Total
19-07-ACH	40	0	40
19-09-ACH	41	0	41

Table 5: Returned results in the 2019 ERNDIM ACDB London scheme.

Submissions	Number of laboratories	%
2	39	88.6
1	3	6.8
0	2	4.6

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 2019 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, 2019).

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, 2019.

Table 7: Samples eligible for critical errors in the 2019 ERNDIM ACDB London

Sample	Critical errors
ACDB-UL-2019-A	0
ACDB-UL-2019-B	0
ACDB-UL-2019-D	1

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.

8. Proficiency of the 2019 surveys

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

In 2019, 39 participants submitted 2 reports including 0 educational participants. From the 39 ordinary (non-educational) participants 37 (95%) achieved satisfactory performance (score ≥ *17*, no critical error). 7 participants did not accomplish satisfactory performance, including 5 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 2019 surveys.

Sample ID	Sample type	Proficiency (%)
ACDB-UL-2019-A	Isovaleric acidaemia	98.8
ACDB-UL-2019-B	Methylmalonic acidaemai	98.1
ACDB-UL-2019-C	Carnitine palmitoyl transferase type 2	59.4
ACDB-UL-2019-D	Propionic acidaemia	97.0
ACDB-UL-2019-E	MADD (acute neonatal)	82.3
ACDB-UL-2019--F	MADD (adult mild)	89.6

7 Performance Support letters will be sent for the 2019 surveys. None of these 7 participants have also received a performance support letter in 2018 or 2017. 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 2018 scheme 2 Performance Support letters were sent.

9. Results of individual samples and evaluation of reporting

ACDB-UL-2019-A. All respondents correctly reported significantly raised isovaleryl (C5) carnitine in this sample, and suggested appropriate tests to exclude other caused of a raised C5 carnitine: 2-methylbutyryl carnitine or pivaloyl carnitine, and confirm the diagnosis.

ACDB-UL-2019-B. All respondents found elevated propionyl carnitine in this sample, although only about half noted a raised methylmalonyl carnitine. All suggested a defect in propionate metabolism and described appropriate second line tests to refine the diagnosis.

ACDB-UL-2019-C. This sample presented major difficulties in interpretation to many participants. Carnitine supplementation meant that the profile was not typical of untreated CPT2, although the acetyl (C2) carnitine was lower than would be expected for the level of free carnitine, and the long chain acylcarnitines were close to or above the upper reference limit. Upper reference limits for (C16+C18:1)/C2 vary widely between centres. This is likely to reflect the difficulties with whole blood as a matrix: a reference limit low enough to detect most cases will generate very large numbers of false positives, likewise a high limit may result in missed diagnoses, particularly in well patients. The relatively high long chain acylcarnitines (C16, C18, C18:1 and C18:2) should have alerted those who suggested CPT1 on the basis of a raised C0/(C16+C18) ratio that this diagnosis was unlikely. Heiner-Fokkema, M. R., et al. (2017) JIMD reports **32**: 33-39, showed that, in plasma at least, C18:1 carnitine was lower than <0.05 µmol/l in patients with CPT1. It was decided that this sample would be included in the scoring following discussions in the ERNDIM scientific advisory board.

ACDB-UL-2019-D. All but one of the respondents correctly reported significantly raised propionyl (C3) carnitine in this sample, and suggested appropriate tests to clarify and confirm the location of the deficiency in the propionate pathway. Failure to recognise the abnormality in this sample constituted a "Critical Error"

ACDB-UL-2019-E. All respondents found low free carnitine in this sample. 35/41 also found elevated C4, C5DC, C14:1, C5, C10, C12, C14 or C16:1, with a majority commenting on more than one raised acylcarnitine. Only 21/40 included MADD in their differential diagnosis. This was clearly a challenging sample due to the carnitine depletion.

ACDB-UL-2019-C. All respondents found the elevated C8 and C10 acylcarnitines in this sample. There was some divergence in interpretation between those excluding MCADD on the basis of the normal C8/C10 ratio and normal C10:1 acylcarnitine, and those suggesting MCADD because of the raised C8 carnitine. However most respondents suggested appropriate follow-up tests to clarify/confirm the diagnosis.

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 2019.

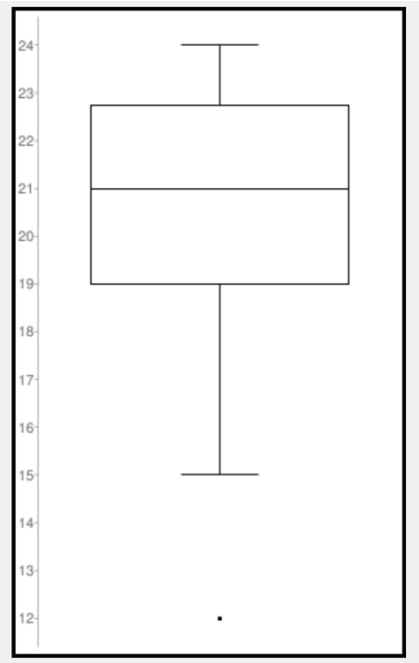
The anonymous data are accessible to all participants. Individual data are only visible to your laboratory.

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
1					4	4	3	11	11	PS
2	4	4	3	11	4	4	4	12	23	
3	4	4	1	9	4	3	3	10	19	
4	4	4	4	12	4	4	4	12	24	
5	4	4	3	11	0	3	3	6	17	CE
6										NS
7	4	4	4	12	4	4	3	11	23	

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
8	4	4	3	11	4	3	3	10	21	
9	4	3	1	8	4	3	3	10	18	
10	4	4	4	12	4	2	4	10	22	
11	4	4	3	11	4	3	3	10	21	
12	4	4	4	12	3	3	4	10	22	
13	4	4	1	9	4	3	4	11	20	
14	4	4	1	9	4	3	4	11	20	
15	4	4	3	11	4	3	4	11	22	
16	4	4	1	9	4	3	4	11	20	
17	4	4	2	10	4	4	3	11	21	
18	4	4	4	12	4	4	4	12	24	
19	3	3	0	6	4	3	4	11	17	
20	4	4	4	12	4	4	4	12	24	
21										NS
22					4	2	3	9	9	PS
23	4	4	3	11	4	2	4	10	21	
24	4	4	2	10					10	PS
25	4	4	4	12	4	4	4	12	24	
26	4	4	2	10	4	4	3	11	21	
27	4	4	4	12	4	4	4	12	24	
28	4	4	1	9	4	4	4	12	21	
29	4	4	4	12	4	4	4	12	24	
30	4	4	3	11	4	3	2	9	20	
31	4	4	2	10	4	2	3	9	19	
32	4	4	1	9	4	4	4	12	21	
33	4	4	0	8	4	3	4	11	19	
34	4	4	4	12	4	4	4	12	24	
35	4	4	0	8	4	2	3	9	17	
36	4	4	3	11	4	3	4	11	22	
37	4	4	1	9	4	4	4	12	21	
38	4	4	1	9	4	4	4	12	21	
39	4	4	2	10	4	4	3	11	21	
40	3	3	1	7	4	1	3	8	15	PP
41	4	4	3	11	4	3	4	11	22	
42	4	4	3	11	4	4	4	12	23	
43	4	4	2	10	4	3	3	10	20	
44	4	4	3	11	4	4	4	12	23	

*) 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



11. Preview of the scheme in 2020

The format of the ACDB 2020 scheme will be similar to that of previous years.

Changes planned for 2020:
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