ERNDIM
Acylcarnitines in DBS
Rome

ANNUAL REPORT 2020

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1. Introduction

The ERNDIM Acylcarnitine in dried blood spots scheme offers dried blood spots obtained from patients with confirmed diagnoses. The scheme is focused to let the participating laboratories will gain or maintain experience to identify organoacidopathies and fatty acid β-oxidation defects. The scheme is organised by Dr. Cristiano Rizzo – Laboratory of Metabolic Diseases Bambino Gesù Children's Hospital -Rome 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 a 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 because identify a normal profile, as such, is as important as correctly identifying abnormal ones. Correctly identifying a profile as normal can avoid unnecessary further investigation and distress to the patient and family.

2. Participants

In 2020 45 laboratories from many different countries participated in the ACDB Rome scheme. No laboratories were educational participants in 2020. They took part in all aspects of the scheme and will receive interim reports with scores, but performance is not indicated on the ERNDIM certificate of performance.

Participants and new applicants will be 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.

[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

Table 1: Geographical distribution of participants				
Country	*Number of laboratories*		*Country*	*Number of laboratories*
BELGIUM	5		MALAYSIA	3
BULGARIA	1		MOROCCO	1
CROATIA	1		PORTUGAL	3
CZECH REPUBLIC	2		REPUBLIC OF SINGAPORE	1
GERMANY	1		SLOVAKIA	2
GREECE	1		SLOVENIA	1
ISRAEL	2		UK	1
KINGDOM of SAUDI ARABIA	1		SPAIN	8
KUWAIT	1		SWITZERLAND	2
LEBANON	1		TAIWAN	1
LITHUANIA	1		USA	5

3. Design of the scheme and logistics

As usual, the samples used in 2020 were authentic human blood spot samples, 6 from affected patients. Sample C is a common sample among the three schemes (Rome, Heidelberg and London).
All samples selected by the Scientific Advisor are prepared from 30-50µl of lithium heparin anticoagulated whole blood on 903 Whatmann 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 Rome scheme.

	1st Submission Round	**2nd Submission Round**
Sample ID's:	ACDB-IR-2020-A ACDB-IR-2020-B ACDB-IR-2020-C	ACDB-IR-2020-D ACDB-IR-2020-E ACDB-IR-2020-F
Shipment of samples	February 11th, 2020	
Start of analysis (clinical data available)	March 9th, 2020	June 8th, 2020
Reminder for result submission	June 20th, 2020	June 22th, 2020
Results submission deadline:	June 26 th, 2020	June 29nd, 2020
Interim reports available on CSCQ website	July 30th, 2020	August 08th, 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 Rome scheme.

Survey	Sample no.	Diagnosis
20-03-ACR	ACDB-IR-2020-A	Carnitine Acylcarnitine translocase deficency
	ACDB-IR-2020-B	Medium chain Acyl-CoA dehydrogenase deficency
	ACDB-IR-2020-C	Long Chain 3-hydroxyacyl-CoA dehydrogenase deficency
20-06-ACR	ACDB-IR-2020-D	Ethylmalonic encephalopathy (ETHE1)
	ACDB-IR-2020-E	Propionyl-CoA carboxylase deficency
	ACDB-IR-2020-F	Succinyl-CoA ligase subunit beta (SUCLA2)

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: Returned results in the 2020 ERNDIM ACDB Rome scheme.

Submissions	Number of laboratories	%
2	38	84.4
1	3	6.6
0	4	8.8

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 in Table 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 19st, 2020).

Table 5: 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 19st, 2020.

Table 6: Samples eligible for critical errors in the 2020 ERNDIM ACDB Rome

Sample	Critical errors
ACDB-IR-2020-B	1
ACDB-IR-2020-C	4

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'.

Sample ACDB-IR-2020-A and ACDB-IR-2020-F were considered as educational samples.

Satisfactory performance is defined as **70% of maximum score** which equates **11/16** 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, 38 participants submitted 2 reports. From the 45 ordinary participants 33 (73%) achieved satisfactory performance (score ≥ *11*, no critical error). 8 participants did not accomplish satisfactory performance, including 1 poor performer and 7 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 7.

Table 7: Overall proficiencies of the 2020 surveys.

Sample ID	Sample type	Proficiency (%)
ACDB-IR-2020-A	Carnitine Acylcarnitine translocase deficency	Educational
ACDB-IR-2020-B	Medium chain Acyl-CoA dehydrogenase deficency	97.6
ACDB-IR-2020-C	Long Chain 3-hydroxyacyl-CoA dehydrogenase deficency	89.0
ACDB-IR-2020-D	Ethylmalonic encephalopathy (ETHE1)	90.8
ACDB-IR-2020-E	Propionyl-CoA carboxylase deficency	100
ACDB-IR-2020-F	Succinyl-CoA ligase subunit beta (SUCLA2)	Educational

7 Performance Support letters will be sent for the 2020 surveys. 4 of these *7* participants have also received a performance support letter in 2019. 5 Critical Errors letters will be sent for the 2020 surveys. 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 ACDB-IR-2019 survey 4 Performance Support letters were sent.

9. Results of individual samples and evaluation of reporting

Sample ACDB-IR-2020 A

19 year old, male. Patient admitted at the age of 3 days for vomit, hypoglycemia, hyperammonemia and hepatic dysfunction. In treatment.

Known diagnosis: **Carnitine Acylcarnitine translocase deficiency (OMIM 212138)**

Analytical details and interpretation: Significant increase was found in long chain acylcarnitines (from C14 to C18), medium chain acylcanitines and C4-carnitine.

9/41 (22%) respondents considered CACT o CPT2 deficiency as the most likely diagnosis.

Therefore this sample is considered an educational sample

Sample ACDB-IR-2020 B

Patient details: 3 year old, female. Diagnosed at birth. Patient admitted for state of drowsiness. In treatment.

Known diagnosis: **medium chain acyl-CoA dehydrogenase deficiency (OMIM 201450)**

Analytical details and interpretation: Significant increases were found in C6, C8, C10:1, acylcarnitine species. The most used ratio were C8/C2 and C8/C10.

40/41 (97%) respondents considered Medium Chain Acyl-CoA Dehydrogenase deficiency (MCAD) as the most likely diagnosis and 8 respondents considered as an alternative diagnosis Multiple Acyl-CoA Dehydrogenase deficiency (MADD). 1 respondent considered CPT1 deficiency as the most likely diagnosis.
The diagnosis of CPT1 deficiency is considered a critical error

Sample ACDB-IR-2020 C

15 year old, male. Patient admitted at the age of 4 months for hypotonia, hypoglycemia, hypocalcemia and myoglobinuria. In treatment.

Known diagnosis: **Long Chain 3-Hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) (OMIM 609016)**

Analytical details and interpretation: 38/40 (95%) respondents reported an increase of at least 2 or more long chain hydroxyl acylcarnitine species. Significant increases were found in C16OH, C18OH and C18:1OH hydroxyl acylcarnitine species.

35/41 (85%) respondents considered both Long Chain 3-Hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) and Mitochondrial trifunctional protein deficiency (MTP) as the most likely diagnosis, 4/41 suggested Isovaleryl-CoA dehydrogenase deficiency (IVA) or 2-Methybutyryl-CoA dehydrogenase deficiency (2MBD/SBCAD).

The diagnosis of Isovaleryl-CoA dehydrogenase deficiency (IVA) or 2-Methybutyryl-CoA dehydrogenase deficiency (2MBD/SBCAD) are considered a critical error

Sample ACDB-IR-2020 D

Patient details: 6 year old, female. Diagnosed at the age of 6 months. Patient admitted for acidosis, encephalopathy, acranocyanosis and psychomotor retardation. Liver transplant at 9 months.

Known diagnosis: **ethylmalonic encephalopathy (EE) caused by mutation in the ETHE1 gene (OMIM 602473)**

Analytical details and interpretation: 36/38 (94%) respondents reported a significant increase of C4-carnitine levels, 34/38 (89%) respondents reported a significant increase of C5-carnitine levels, 7 noted a significant elevation of C6-carnitine, 11 noted a significant elevation of C8-carnitine, 8 noted a significant elevation of C10-carnitine, 5 noted a significant elevation of C14:1-carnitine. The increased ratios were C4/C2, C4/C3, C5/C2, C5/C3, C8/C2

27/38 (71%) respondents considered Ethylmalonic encephalopathy (EE), 8 respondents suggested Multiple dehydrogenase deficiency (Glutaric acidemia type 2), 2 respondents suggested isovaleric acidemia, 1 respondent suggested 2-Methylbutyryl-CoA dehydrogenase deficiency as the most likely diagnosis.

The alternative differential diagnosis suggested by respondents included:
a) Multiple dehydrogenase deficiency (Glutaric academia type 2) (n=11)
b) Short –Chain Acyl-CoA dehydrogenase deficiency (SCAD) (n=11)
c)Isovaleryl-CoA dehydrogenase deficiency (n=5)
d) Isobutyryl-CoA dehydrogenase deficiency (n=6)
e) 2-Methylbutyryl-CoA dehydrogenase (n=2)
f) Ethylmalonic encephalopathy (EE) (n=5)

Sample ACDB-IR-2020 E

Patient details: 18 year old, male. Diagnosed at the age of 11 years. Patient admitted for dilated cardiomyopathy. In treatment

Known diagnosis: **Propionyl-CoA carboxylase deficiency, (OMIM 606054)**

Analytical details and interpretation: 38/38 (100%) respondents reported a significant increase of C3 (propionylcarnitine) levels, 19 noted a significant elevation of C3/C2 ratio, 10 noted a significant elevation of C3/C16 ratio, 4 noted a significant elevation C3/C0 ratio, 6 noted a significant elevation of C3/methionine ratio.
9 respondents comment that the C4DC concentration (methylmalonylcarnitine+succinylcarnitine) was normal and 1 respondent comment that C4DC concentration was elevated.
2 respondents performed a II tier test which provided measurement of 3-hydroxy-propionate, methylmalonate, methylcitrate and homocysteine on the blood spot. MMA were found to be normal

All 38/38 (100%) respondents considered a disorder of the propionate pathway as the most likely diagnosis, 5 of these specified just a propionic acidemia, 31/38 included methylmalonic and propionic acidemias and 9 of them included defects in B12 synthesis and transport, 2/38 included Succinyl-CoA synthase deficiency as part of their differential diagnosis.

Sample ACDB-IR-2020 F

Patient details: 15 year old, male. Patient admitted at the age of 7 months for encephalopathy, psychomotor retardation and hypotonia. In treatment

Known diagnosis: **Succinyl-CoA ligase subunit beta (SUCLA2 OMIM 603921)**

Analytical details and interpretation: 19/38 (50%) respondents reported an increase of C4DC (succinylcarnitine/methylmalonylcarnitine).

19/38 (50%) respondents considered Succinate-CoA ligase deficiency (SUCLA2 or SUCLAG1), 2 respondents suggested primary carnitine deficiency, 2 respondents suggested methylmalonic acidemia, 2 respondents suggested 3-methycrotonyl-CoA carboxylase deficiency, 1 suggested multiple carboxylase deficiency, 1 suggest a valproate therapy profile and as the most likely diagnosis.
11 respondents considered a normal acylcanitine profile.

The alternative differential diagnosis suggested by respondents included:
a) Methylmalonic acidemia (n=5)
b) 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG) (n=3)
c) Beta-ketothiolase deficiency (n=2)
d) 2-methyl 3-hydroxybutyryl-CoA dehydrogenase deficiency (2M3HBA) (n=4),
e) 3- methylglutaconic aciduria type I (3-methylglutaconyl-CoA hydratase deficiency), and/or other 3-methylglutaconic aciduria (3MGA) (n=6)
f) Biotine deficiency (n=3)
g) 3-methylcrotonyl-CoA carboxylase (3MCC) deficiency (n=7)
h) Mitochondrial DNA depletion (n=2)
i) Valproate treatment (n=1)

This sample is considered an educational sample.

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.

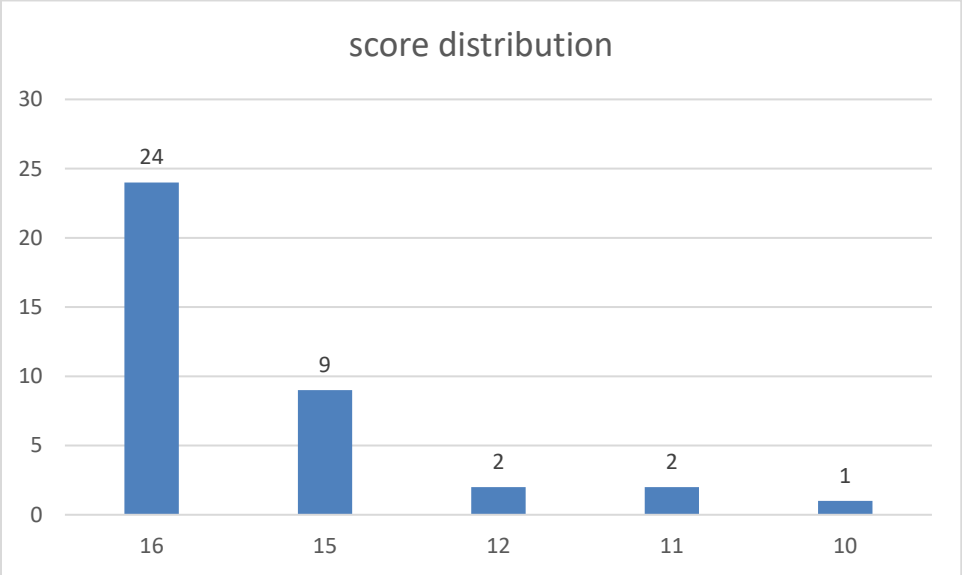
The anonymous data are accessible to all participants. Individual data are only visible to your laboratory.

lab #	A	B	C	sum	D	E	F	SUM	tot score	performance
1	edu	4	4	8	4	4	edu	8	16	
2	edu	4	4	8	4	4	edu	8	16	
3	edu	4	4	8	4	4	edu	8	16	
4	edu	4	4	8	4	4	edu	8	16	
5	edu	4	4	8	4	4	edu	8	16	
6	edu	4	4	8	3	4	edu	7	15	
7	edu	4	4	8	3	4	edu	7	15	
8	edu	4	3	7	4	4	edu	8	15	
9	edu						edu			NS
10	edu	4	4	8	4	4	edu	8	16	
11	edu	4	4	8	4	4	edu	8	16	
12	edu	4	2	6	2	4	edu	6	12	CE
13	edu	4	4	8	4	4	edu	8	16	
14	edu	4	4	8	4	4	edu	8	16	
15	edu	4	4	8	4	4	edu	8	16	
16	edu	4	4	8	3	4	edu	7	15	
17	edu	4	4	8	4	4	edu	8	16	
18	edu	4	4	8	4	4	edu	8	16	

19	edu	4	4	8	4	4	edu	8	16	
20	edu	4	4	8	4	4	edu	8	16	
21	edu	4	0	4	3	4	edu	7	11	CE
22	edu	4	4	8	3	4	edu	7	15	
23	edu	2	4	6	2	4	edu	6	12	CE
24	edu	4	4	8	4	4	edu	8	16	
25	edu	4	4	8	3	4	edu	7	15	
26	edu						edu			NS
27	edu	4	4	8	3	4	edu	7	15	
28	edu	4	4	8	4	4	edu	8	16	
29	edu	4	4	8	4	4	edu	8	16	
30	edu	4	4	8			edu		8	PARS
31	edu	4	4	8	3	4	edu	7	15	
32	edu	4	4	8	4	4	edu	8	16	
33	edu	4	4	8	4	4	edu	8	16	
34	edu	4	4	8			edu	0	8	PARS
35	edu	4	4	8	3	4	edu	7	15	
36	edu	4	0	4	3	4	edu	7	11	CE
37	edu	4	4	8	4	4	edu	8	16	
38	edu	4	4	8	4	4	edu	8	16	
39	edu	4	4	8	4	4	edu	8	16	
40	edu	4	4	8	4	4	edu	8	16	
41	edu	4	4	8	4	4	edu	8	16	
42	edu	4	4	8			edu		8	PARS
43	edu						edu	0		NS
44	edu	4	0	4	2	4	edu	6	10	CE+PS
45	edu						edu	0		ns

NS: Non-submitter
PARS: Partial submitter
CE: Critical error
PS: Poor score

Figure 1: Scores distribution (who submitted results for both rounds)



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.

November 30, 2020

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Dr. Sara Boenzi
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Please note:
This annual report is intended for participants of the ERNDIM QLOU 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	22 June 2021	2020 annual report published