ERNDIM Acylcarnitines in DBS Rome

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 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 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 2019 47 laboratories from many different countries participated in the ACDB Rome scheme. No laboratories were educational participants in 2019 (1 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 Rome 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*
BELGIUM	6		MALAYSIA	3
BULGARIA	1		MOROCCO	1
CROATIA	1		PORTUGAL	3
CZECH REPUBLIC	2		REPUBLIC OF SINGAPORE	1
FRANCE	1		SLOVAKIA	2
GREECE	1		SLOVENIA	1
ISRAEL	3		SOUTH AFRICA	1
KINGDOM of SAUDI ARABIA	1		SPAIN	8

Table 1: Geographical distribution of participants				
Country	*Number of laboratories*		*Country*	*Number of laboratories*
KUWAIT	1		SWITZERLAND	2
LEBANON	1		TAIWAN	1
LITHUANIA	1		UK	2
UNITED ARAB EMIRATES	1		USA	2

3. Design of the scheme and logistics

As usual, the samples used in 2019 were authentic human blood spot samples, 5 from affected patients and 1 from healthy individuals.
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 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 Rome scheme.

	1st Submission Round	**2nd Submission Round**
Sample ID's:	ACDB-IR-2019-A ACDB-IR-2019-B ACDB-IR-2019-C	ACDB-IR-2019-D ACDB-IR-2019-E ACDB-IR-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	July 26th, 2019	October 09th, 2019

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 Rome scheme.

Survey	Sample no.	Diagnosis
19-07-ACR	ACDB-IR-2019-A	Glutaric acidemia type I
	ACDB-IR-2019-B	Medium chain Acyl-CoA dehydrogenase deficency
	ACDB-IR-2019-C	normal
19-09-ACR	ACDB-IR-2019-D	Propionic acidemia
	ACDB-IR-2019-E	Isovaleric acidemia
	ACDB-IR0-2019-F	3-Methyl-crotonyl-CoA Carboxylase deficency

The scheme format was kept identical to those of previous years. In most instances the samples were shipped by courier unless otherwise requested by the participating laboratory. 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 2019 ERNDIM ACDB Rome scheme.

Submissions	Number of laboratories	%
2	41	87
1	3	6
0	3	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 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 21st, 2019.

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

Sample	Critical errors
ACDB-IR-2019-A	0
ACDB-IR-2019-B	0
ACDB-IR-2019-D	0
ACDB-IR-2019-E	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 **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, 41 participants submitted 2 reports. From the 47 ordinary (non-educational) participants 41 (87%) achieved satisfactory performance (score ≥ *17*, no critical error). 6 participants did not accomplish satisfactory performance, including 6 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-IR-2019-A	Glutaric acidemia type I	99.4
ACDB-IR-2019-B	Medium chain Acyl-CoA dehydrogenase deficency	99.4
ACDB-IR-2019-C	normal	94.6
ACDB-IR-2019-D	Propionic acidemia	98.8
ACDB-IR-2019-E	Isovaleric acidemia	99.4
ACDB-IR-2019-F	3-Methyl-crotonyl-CoA Carboxylase deficency	76.7

6 Performance Support letters will be sent for the 2019 surveys. 3 of these *6* 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 6 Performance Support letters were sent.

9. Results of individual samples and evaluation of reporting

Sample ACDB-IR-2019 A

Patient details: 3 year old male. Patient admitted for vomiting and hypertonia. Diagnosis at the age of 3 months.

Known diagnosis: **glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I ; OMIM 231670).**

Analytical details and interpretation: Significant increase was found in glutarylcarnitine (C5DC) this was reported by 43/43 respondents (100%). The most used ratio were C5DC/C16, C5DC/C8 and C5DC/C5OH
43/43 (100%) respondents considered glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I) as the most likely diagnosis.

Sample ACDB-IR-2018 B

Patient details: 8 year old male. Diagnosed at birth. Patient admitted for hypoglycaemia, hyperammonemia and acidosis In treatment.

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

Analytical details and interpretation: Significant increase was found in C6-carnitine, C8-carnitine, C10:1-carnitine and C8/C10, C8/C2 ratios.
43/43 (100%) respondents considered Medium Chain Acyl-CoA Dehydrogenase deficiency (MCAD) as the most likely diagnosis and 5 respondents considered as an alternative diagnosis Multiple Acyl-CoA Dehydrogenase deficiency (MADD).

Sample ACDB-IR-2019 C

Patient details: 30 year old female, Patient admitted for muscle pain. This sample was from an adult after heavy workout in the gym.

Known diagnosis: normal

Analytical details and interpretation: 38/41 (92%) respondents reported a **normal acylcarnitines profile**. One respondent considered Carnitine palmitoyltransferase II (CPT2), one respondent considered Primary carnitine deficiency an another respondent considered 3-Methylcrotonyl-CoA deficiency as the most likely diagnosis.
Five respondents suggested as a second choice of diagnosis Carnitine Palmitoyltransferase II (CPT2).
One respondent suggested as a second choice of diagnosis adenosine monophosphate dehydrogenase deficiency or glycocen storage disease type V.
One respondent suggested as a second choice of diagnosis Primary Carnitine deficiency

Sample ACDB-IR-2019 D

Patient details: 18 year old female diagnosed at 1 month. Patient admitted for hyperammonemia and acidosis In treatment.

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

Analytical details and interpretation: Significant elevation of C3 (propionylcarnitine), C3/C2 and C3/C16 ratios. Normal C4DC
43/43 (100%) respondents considered a disorder of the propionate pathway as the most likely diagnosis**.**

Sample ACDB-IR-2019 E

Patient details: 20 year old male diagnosed at 8 days. Patient admitted for vomit, hyperammonemia and acidosis In treatment.

Known diagnosis: **Isovaleryl-CoA dehydrogenase deficiency deficiency (OMIM 243500)**

Analytical details and interpretation: 43/43 (100%) respondents reported an increase of C5-Carnitine. Significant increases were found in C5/C2, C5/C0 and C5/C3 ratios.. All respondents considered Isovaleryl-CoA dehydrogenase deficiency or 2-methyl-butyryl-CoA Dehydrogenase deficiency (Short branched chain acyl-CoA dehydrogenase deficiency; SBCAD) as the most likely diagnosis.
Genetic confirmation is a fundamental part of the diagnosis and follow-up of isovaleric acidemia because mild and potentially asymptomatic phenotype variants have been described. Patients who have at least one copy of a c.932C>T (p.A282V) mutant allele can exhibit a mild phenotype or be free of symptoms throughout childhood. (see reference 1)

Reference:

1) A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening.Ensenauer R, Vockley J, Willard JM, Huey JC, Sass JO, Edland SD, Burton BK, Berry SA, Santer R, Grünert S, Koch HG, Marquardt I, Rinaldo P, Hahn S, Matern D.
Am J Hum Genet. 2004 Dec;75(6):1136-42. Epub 2004 Oct 14. DOI: 10.1086/426318

Sample ACDB-IR-2019 F

Patient details: 5 months old asymptomatic male. An increase was found in C5-Hydroxy-carnitine (C5OH) this was mentioned by 33/43 respondents (76%). The most used ratios were C5OH/C8 and C5OH/C0.

Known diagnosis: **3-methyl-crotonyl-CoA carboxylase deficiency** (**OMIM 210200)**.

Analytical details and interpretation: 31/43 (72%) respondents considered 3-methyl-crotonyl-CoA carboxylase deficiency (3MCC) as the most likely diagnosis.

Causes of elevated C5OH-carnitine include Biotinidase deficiency and various organic acidemias (HMG, BKT, 3MCC, MCD, 3MGC, 3-MCC (mat), 2M3HBA and MT-ATP6).

The differential diagnosis suggested by 17 respondents included:

a) 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG) (n=15)
b) Beta-ketothiolase deficiency (n=17)
c) 2-methyl 3-hydroxybutyryl-CoA dehydrogenase deficiency (2M3HBA) (n=15),
d) 3- methylglutaconic aciduria type I (3-methylglutaconyl-CoA hydratase deficiency), and/or other 3-methylglutaconic aciduria (3MGA) (n=17)
e) Biotine deficiency (n=3)
f) multiple carboxylase deficiency (MCD) including biotinidase deficiency and holocarboxylase synthetase deficiency(n=17)
g) 3-methylcrotonyl-CoA carboxylase (3MCC) deficiency of the mother (n=5)
h) MT-ATP6 (n=1)
i) Valproate treatment (n=1)

2 respondents considered glutaryl-CoA dehydrogenase deficiency as the most likely diagnosis based on elevated value of C5DC (glutarylcarnitne)

43 respondents suggested these follow up test to confirm the diagnosis.
22 respondents suggested the mutation analysis of the MCCC1 and MCCC2 genes in the patient and 5 respondents suggested carrying out the mutation analysis also on the mother.

5 respondents suggested mutation study of the biotinidase gene, 2 respondents suggested NGS panel or other appropriate gene mutation analysis guided by the results of the urinary organic acids analysis (n=33).

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.

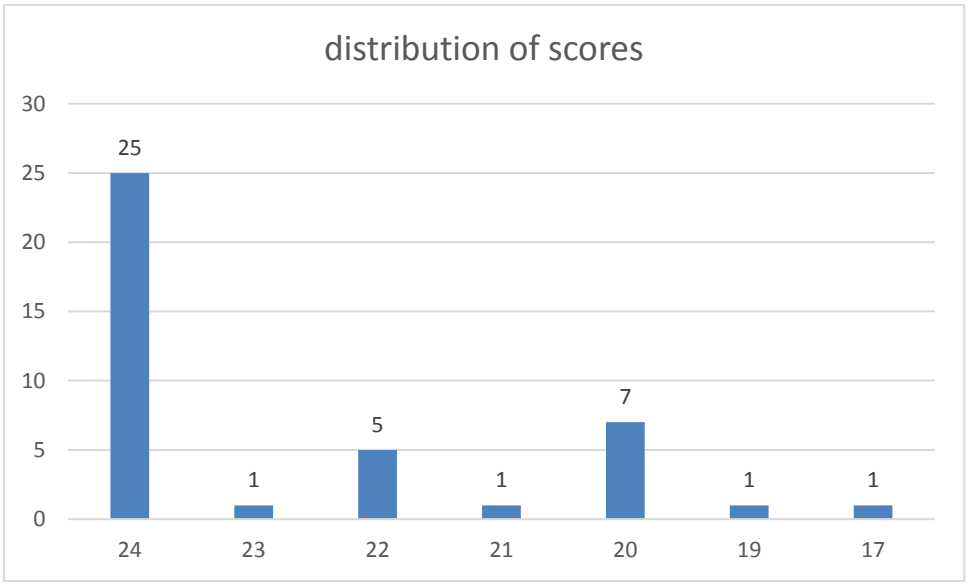
The anonymous data are accessible to all participants. Individual data are only visible to your laboratory.

Lab n°	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	0	8	20	
3	4	4	4	12	4	4	4	12	24	
4	4	4	4	12	4	4	1	9	21	
5	4	4	4	12	4	4	2	10	22	
6	4	4	4	12	4	4	4	12	24	
7	4	4	4	12	4	4	4	12	24	
8	4	4	4	12	4	4	4	12	24	
9										NS
10	4	4	2	10	4	4	4	12	22	
11	4	4	4	12	4	4	0	8	20	
12	4	4	4	12	4	4	2	10	22	
13	4	4	1	9	4	4	0	8	17	
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	4	4	4	12	4	4	4	12	24	
19	4	4	4	12	4	4	0	8	20	
20	4	4	4	12	4	4	4	12	24	
21	4	4	4	12	4	4	0	8	20	
22	4	4	4	12	4	4	2	10	22	
23	3	3	4	10	3	3	3	9	19	
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	4	4	4	12	4	4	4	12	24	
28										NS
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	4	4	4	12	24	
32	4	4	4	12	4	4	0	8	20	

33					4	4	4	12	12	PS
34	4	4	4	12	3	4	4	11	23	
35	4	4	4	12	4	4	2	10	22	
36	4	4	4	12	4	4	4	12	24	
37	4	4	4	12	4	4	4	12	24	
38	4	4	4	12					12	PS
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	12	PS
42	4	4	4	12	4	4	4	12	24	
43	4	4	4	12	4	4	4	12	24	
44	4	4	4	12	4	4	0	8	20	
45										NS
46	4	4	4	12	4	4	4	12	24	
47	4	4	0	8	4	4	4	12	20	

NS: Non-submitter
PS: Parcial submitter

Figure 1: Distribution of scores (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.

January 21, 2020

Dr. Cristiano Rizzo

Laboratory of metabolic disease

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