ERNDIM
Acylcarnitines in DBS *Rome*
ANNUAL REPORT 2018

Scientific Advisor	**Website for reporting results**	**Administration office:**
Dr. Cristiano Rizzo Laboratory of metabolic disease (lab n°2031) Bambino Gesù Children's Hospital Department of Metabolism Viale di s. Paolo 15 00165 Roma -Italy Tel +39-0668592519 Fax +39-0668593009 *e-mail cristiano.rizzo@opbg.net*	Dr. Xavier Albe CSCQ Swiss Center for Quality Control 2 chemin du Petit-Bel-Air CH-1225 Chêne-Bourg Switzerland e-mail : Xavier.Albe@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

January 18th 2019
v2 July 19th 2019[1]

1. Introduction

The ERNDIM Acylcarnitine in dried blood spots scheme offers dried blood spots from patients with known acylcarnitine disorders. 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.

2. Participants

In 2018 *43* laboratories from many different countries participated in the ACDB *Rome*. One laboratory was an educational participants 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.

3. Design of scheme and logistics

The scheme has been designed and planned by Cristiano Rizzo as Scientific Advisor and distributed by Dr Xavier Albe as Scheme Organiser, both were appointed by and according to procedures laid down by the ERNDIM Board.

All EQA materials are 30-50µl of lithium heparin anticoagulated whole blood dried as blood spots on Perkin Elmer (Ahlstrom) 226 paper. All samples are obtained following local ethical and consent guidelines.

Six samples (2018.01- 2018.06) were sent out to the 43 laboratories from 23 countries worldwide assigned to the *Rome* centre of the ERNDIM dried blood spot acylcarnitine scheme. The first samples were sent out in May 5th, 2018, with a return date of June 25th, 2018 for samples ACDB-RM-2018-A, ACDB-RM-2018-B ACDB-RM-2018-C and a second return date of September 17th for samples ACDB-RM-2018-D, ACDB-RM-2018-E, ACDB-RM-2018-F.

Table 1. Samples included in the 2018 ERNDIM ACDB *Rome* scheme. Sample ACDB-RM-2018-B was donated by Dr Christine Vianey-Saban Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal Centre de Biologie et de Pathologie Est, Lion-France ; sample ACDB-RM-2018-F was donated by Dra. Begoña Merinero Centro de Diagnóstico de Enfermedades Moleculares,Facultad de CienciasUniversidad Autónoma de Madrid-Spain.

Version Number (& Date)	Amendments
[1] version 2 (19th July 2019)	• Page 7: Addition of Scientific Advisor signature to authorization of report.

Samples, reporting deadline	Sample no.	Sample type (diagnosis)
2018-1-3.June 25th, 2018	ACDB-RM-2018-A	LCHAD deficiency
	ACDB-RM-2018-B	Glutaric acidemia type II (MADD)
	ACDB-RM-2018-C	Propionic acidemia
2018-2-6. September 17th 2018	ACDB-RM-2018-D	Short branched chain acyl-CoA dehydrogenase deficiency (SBCAD)
	ACDB-RM-2018-E	Methylmalonic aciduria, Vitamin B12-Responsive, due to defect in synthesis of Adenosylcobalamin, cblA
	ACDB-RM-2018-F	Glutaric acidemia type I

All submitted results are treated as confidential information, and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting.

Participants were asked to submit results online for the first time in 2018 using the result submission website: https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php

4. Scoring of results

In the process of working towards accreditation for ERNDIM there is a need for harmonization of performance assessment within the qualitative schemes (see ERNDIM 'Newsletter Spring 2013' at www.erndim.org). In 2013 we changed the scoring system from the former scale (-2, -1, 0, +1, +2) to the four point system (+1, +2, + 3, +4) which is used also in the DPT schemes. In this system a maximum of two points is given each for analytical results and interpretation, with the latter including suggestions for further testing/actions. The total score achievable for a single circulation of three samples is twelve and twenty four for the whole sample set of six samples per year. To obtain satisfactory performance a score of 16 or more should be achieved on two returns. This increased to 17/24 for 2018. Laboratories that participate only in one circulation are treated as non-submitters.

Table 2. 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

From the 2014 scheme onwards another criteria for satisfactory performance is the absence of any "critical error" which is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient.

Returns for samples circulation of 2018.- A;B;C were received from 41 respondents (95%); 40 of these arrived by the initial due date. For samples circulation 2018.-D;E;F. valid returns were received from 37 (86%) respondents; 37 of these arrived before the due date.

There was 1 laboratory which failed to make a return on both circulations. 4 laboratories failed to make a return only on second circulation.

A summary of the samples sent, and the number of respondents detecting the key acylcarnitines and/or suggesting the definitive diagnosis as part of their differential diagnosis, is given in the table below.

Table 3. Criteria for scoring of diagnostic proficiency of 2018 samples.

Sample	Diagnoses (or combinations of possible diagnoses) scored as correct - 2 points	Combinations of possible diagnoses scored as partially correct - 1 point	Not correct - 0 points
ACDB-RM-2018-A	**35**	**5**	**1**
ACDB-RM-2018-B	**39**	**1**	**1**
ACDB-RM-2018-C	**39**	**2**	**1**
ACDB-RM-2018-D	**36**	**1**	
ACDB-RM-2018-E	**37**		
ACDB-RM-2018-F	36	1	

Starting from the 2014 schemes, the concept of 'critical error' was 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 are sufficient according to the requirement set by the SAB. The classification of samples to be judged for critical error were undertaken at the SAB meeting held on November 28th , 2018. All samples were eligible for critical error.

Failing to report an increase in C5 carnitine in sample ACDB-RM-2018-D is considered a critical error as well as failing to report an increase in C3 carnitine in sample ACDB-RM-2018-E is considered a critical error.

Among the reports of regular participants, **2 critical errors were identified in 2018 by one of the participants**

5. Communication of results

Interim reports with diagnoses, summaries of the results submitted and interim scores were made available September 5th 2018 (samples ACDB-RM-2018-A, ACDB-RM-2018-B and ACDB-RM-2018-C) and *December 7th 2018* (samples ACDB-RM-2018-D, ACDB-RM-2018-E and ACDB-RM-2018-F).

The annual report summarises scheme organisation and results.

ERNDIM provides a single certificate for all its schemes with details of participation and performance.
1 Performance Support letter will be sent for the 2018 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.

6. Proficiency of the 2018 scheme

In 2018, *37* participants submitted 2 reports. Of the *37* ordinary (non-educational) participants (*100*%) achieved satisfactory performance (score ≥*17)*, Only 1 participant presented 2 critical errors. *5* participants did not accomplish satisfactory performance due to incomplete submission of results (i.e. no report or only 1 survey report submitted instead of 2 reports).

Table 4: Proficiency per sample

Sample	No of returns	A (%)	I (%)	Total (%)
ACDB-RM-2018.A	41	96.3	91.5	93.9
ACDB-RM-2018.B	41	97.6	96.3	97.0
ACDB-RM-2018.C	41	98.8	97.6	98.2
ACDB-RM-2018.D	37	97.3	98.6	98.0
ACDB-RM-2018.E	37	95.9	100.0	98.0
ACDB-RM-2018.F	37	98.6	98.6	98.6

Table 5: Cumulative Scores. The maximum score achievable was 24 points.

Total Score	No of labs (who submitted results for both rounds)
24	29
23	5
20	1
19	1
18	1

7. Results of individual samples and evaluation of reporting

Sample ACDB-RM-2018-A 14 year old female. Patient admitted for vomiting and abdominal pain. Diagnosis at the age of 3 years. Patient on a diet and pharmacological treatment. Elevation of C16-OH, C16:1-OH, C18-OH and C18:1-OH acylcarnitines. Significant elevation of ratio C16-OH/C16.
41/41 (100%) respondents reported an increase of at least 2 or more long chain hydroxyl acylcarnitine species. 34/41 respondents considered both Long Chain 3-Hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) and Mitochondrial Trifuncional protein deficiency (MTP) as the most likely diagnosis, 7/41 suggested only Long Chain 3-Hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) and 1/41 suggested Very long chain acyl-CoA dehydrogenase deficiency (VLCADD).
This sample was from a patient with long chain 3-hydroxy acyl-CoA deficiency (LCHAD OMIM 609016)

Sample ACDB-RM-2018 B
27 year old female, Diagnosed at birth. In treatment. Most of respondents commented on raised of short and medium chain acylcarnitines (C4, C5, C6, C8, C10, C12, C14, C14:1). Elevation of glutarylcarnitine (C5DC) was mentioned by 9 respondents (21%) Several ratios were alterated (C4/C2, C4/C3, C5/C0, C5/C2, C5/C3, C6/C2, C8/C2, C10/C2).
39/41 (95%) respondents considered multiple acyl-CoA dehydrogenase deficiency (MADD) as the most likely diagnosis, 1 suggested Medium chain acyl-CoA dehydrogenase deficiency (MCAD) and 1 suggested short chain acyl-CoA dehydrogenase deficiency (SCAD).
This sample was from a patient with multiple acyl Coa dehydrogenase deficiency (MADD; glutaric aciduria tipe II OMIM 231680)

Sample ACDB-RM-2018 C

14 year old male. Patient admitted for lethargy, vomit, ketoacidosis and hyperammonemia. Diagnosis at age of 3 months. Patient in treatment.
41/41 (100%) respondents reported a significant increase of C3 (propionylcarnitine) levels, 16 noted the significant elevated C3/C2 ratio, 7 noted the significant elevated C3/C16 ratio.
9 respondents comment that the concentration of C4DC (methylmalonilcarnitine+succinilcarnitine) was normal while 1 respondent considered the C4DC elevated.
3 respondents commented on raised of C4DC+C5OH (methylmalonylcarnitine + succinylcarnitine + 3-hydroxy-isovalerylcarnitine) while 1 respondent considered the concentration of C4DC+C5OH normal.
9 respondents commented on raised of C0 (free carnitine) while 6 respondents considered the concentration of C0 normal.
All 41 respondents considered a disorder of the propionate pathway as the most likely diagnosis, 35 of these specified a propionic academia. 37/41 included methylmalonic acidemia, 3/41 included multiple carboxylase deficiency or biotine defect as part of their differential diagnosis.
This sample was from a patient with propionyl-CoA carboxylase deficiency (PA OMIM 606054)

Sample ACDB-RM-2018.D 9 year old male. Asintomatic patient. Significant elevation of C5-carnitine and C5/C0, C5/C2 C5/C3 ratios.
36/37 (94%) respondents reported an increase of C5-Carnitine. Significant increases were found in C5/C2 and C5/C0 ratios.
37/37 (100%) respondents considered **2-methyl-butyryl-CoA Dehydrogenase deficency or Isovaleryl-CoA dehydrogenase deficiency** as the most likely diagnosis, Just 13/38 (34%) respondent suggested in addition, the possibility of false positives due to contamination by pivalate-containing drugs or cosmetic products containing neopentanoate (see reference 1 and 2)
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 3)
This sample was from a patient with Short branched chain acyl-CoA dehydrogenase deficiency (SBCADD OMIM 610006)

Reference:
1) Surprising causes of C5-carnitine false positive results in newborn screening.
Boemer F[1], Schoos R[2], de Halleux V[3], Kalenga M[3], Debray FG[4]. Mol Genet Metab. 2014 Jan;111(1):52-4. doi: 10.1016/j.ymgme.2013.11.005

2) Reducing the False-Positive Rate for Isovalerylcarnitine in ExpandedNewborn Screening: The Application of a Second-Tier Test. Sara Poggiali, BSc, MT1, Daniela Ombrone, BSc1, Giulia Forni, BSc1,Sabrina Malvagia, BSc1, Silvia Funghini, BSc, PhD1, Massimo Mura, Pharm Sc1,Elisabetta Pasquini, MD2, Laura Santoro, BSc3, Vincenzo Bellavia, BSc3, Orazia Maria Granata, BSc3, Cinzia Castana, MD4, Kathleen S. McGreevy, PhD5,Tommaso Silvano Aronica, MD3, and Giancarlo la Marca, Pharm Sc1,6
Journal of Inborn Errors of Metabolism& Screening 2016, Volume 4: 1–7 DOI:10.1177/2326409816661355

3) 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-RM-2018 E
17 year old female, Patient admitted for gastroenteritis, hyperammonemia and acidosis. Diagnosed at age of 16 months. In treatment. Significant elevation of C3 (propionylcarnitine), C3/C2 and C3/C16 ratios. Normal C4DC (methylmalonylcarnitine)
36/37 (97%) respondents reported a significant increase of C3 (propionylcarnitine) levels, 17 noted the significant elevated C3/C2 ratio, 8 noted the significant elevated C3/C16 ratio. 13 respondents comment that the concentration of C4DC (methylmalonilcarnitine+succinilcarnitine) was normal while 2 considered the C4DC elevated.
11 respondents considered the concentration of C0 normal. Just 2 respondents have performed a II tier test which provides measurement of 3-hydroxy-propionate, methylmalonate and homocysteine on the blood spot.
All 37/38 (97%) respondents considered a disorder of the propionate pathway as the most likely diagnosis, 6 of these specified just a propionic acidemia, 1 of these specified just a methylmalonic acidemia, 30/38 included methylmalonic and propionic acidemias and 15 of them included defects in B12 synthesis and transport, 2/38

included multiple carboxylase deficiency and/or biotine defect and/or Succinyl-CoA synthase deficiency as part of their differential diagnosis.
This sample was from a patient with Methylmalonic aciduria, Vitamin B12-Responsive, due to defect in synthesis of Adenosylcobalamin, cblA TYPE OMIM 251100)

Sample ACDB-RM-2018 F
Female investigated at 11 months of age due to macrocephaly. Sample collected at 19 years on treatment. Significant increase was found in glutarylcarnitine (C5DC) was mentioned by 37/37 (100%) respondents (97%). the most used ratio were C5DC/C16, C5DC/C8 and C5DC/C5OH. Ten respondents considered the concentration of C0 normal.
37/37 (100%) respondents considered glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I) as the most likely diagnosis.
This sample was from a patient with Glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I OMIM 231670)

8. Preview of the scheme in 2019

The format of the ACDB 2019 scheme will be similar to that of previous years.

9. Donation of samples

Once again, we are extremely grateful to the centres that can provide informative material for circulation. If any participants can provide samples in the future it would enormously facilitate this scheme, providing, as it does, genuine clinically derived samples for assay and interpretation. 3-4ml of lithium heparin anticoagulated whole blood or 50-60 blood spots of 30-50µl on Whatman (Schleicher & Schuell) 903 or Perkin Elmer 226 paper would provide sufficient material for one circulation. Samples for use in the scheme should be accompanied by a short clinical history and confirmation that informed consent/local ethical approval (as required in the referring centre) for use of the sample has been obtained.

Please contact the ERNDIM Administration office (admin@erndim.org) to discuss possible samples donations. Laboratories donating a sample that is used in the ACDB EQA scheme are eligible for a 20% discount of their participation costs in the ACDB scheme during the following year.

Cristiano Rizzo
Scientific Advisor

Note: *This annual report is intended for the participants of the Acylcarnitines in DBS scheme. The contents of this report or data derived from the use or analysis of ERNDIM EQA materials must not be used in written publications or oral presentations unless the explicit prior consent of ERNDIM has been granted*