ERNDIM
Acylcarnitines in DBS
Heidelberg

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 Claus-Dieter Langhans (metabolic center Heidelberg) 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 44 laboratories from many different countries participated in the ACDB Heidelberg scheme.

One participant sent erroneously results to the Heidelberg scheme. These results were subtracted out from the performance calculations retrospectively.

One laboratory was an educational participant in 2019 (no 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*
Argentina	3		Italy	1
Austria	2		Luxembourg	1
China	4		Mexico	1
France	16		Netherlands	5
Germany	9		Turkey	1
Hong Kong S.A.R.	1			

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 one from a healthy individual.
All samples selected by the Scientific Advisor are prepared from 30-50µl of lithium heparin anticoagulated whole blood on Whatman (Schleicher & Schuell) 903™ 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 Heidelberg scheme.

	1st Submission Round	**2nd Submission Round**
Sample ID's:	ACDB-DH-2019-A ACDB-DH-2019-B ACDB-DH-2019-C	ACDB-DH-2019-D ACDB-DH-2019-E ACDB-DH-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	August 16th, 2019	November 4th, 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 Heidelberg scheme.

Survey	Sample no.	Diagnosis
19-07-ACH	ACDB-DH-2019-A	propionyl-CoA carboxylase deficiency (propionic acidaemia)
	ACDB-DH-2019-B	3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency
	ACDB-DH-2019-C	normal
19-09-ACH	ACDB-DH-2019-D	isovaleric acidaemia
	ACDB-DH-2019-E	multiple acyl-CoA dehydrogenase (MAD) deficiency
	ACDB-DH-2019-F	long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

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 2019 ERNDIM ACDB Heidelberg scheme.

Submissions	Number of laboratories	%
2	41	93
1	-	-
0	3	7

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 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 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 6: Samples eligible for critical errors in the 2019 ERNDIM ACDB Heidelberg

Sample	Critical errors
ACDB-DH-2019-F	3

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 including 1 educational participants. From the 41 ordinary (non-educational) participants 37 (84%) achieved satisfactory performance (score ≥ *17*, no critical error). Four 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 7.

Table 7: Overall proficiencies of the 2019 surveys.

Sample ID	Sample type	Proficiency (%)
ACDB-DH-2019-A	propionyl-CoA carboxylase deficiency (propionic acidaemia)	98
ACDB-DH-2019-B	3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency	85
ACDB-DH-2019-C	normal	83
ACDB-DH-2019-D	isovaleric acidaemia	95
ACDB-DH-2019-E	multiple acyl-CoA dehydrogenase (MAD) deficiency	88
ACDB-DH-2019-F	long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	88

Six Performance Support letters will be sent for the 2019 surveys. None of these six 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

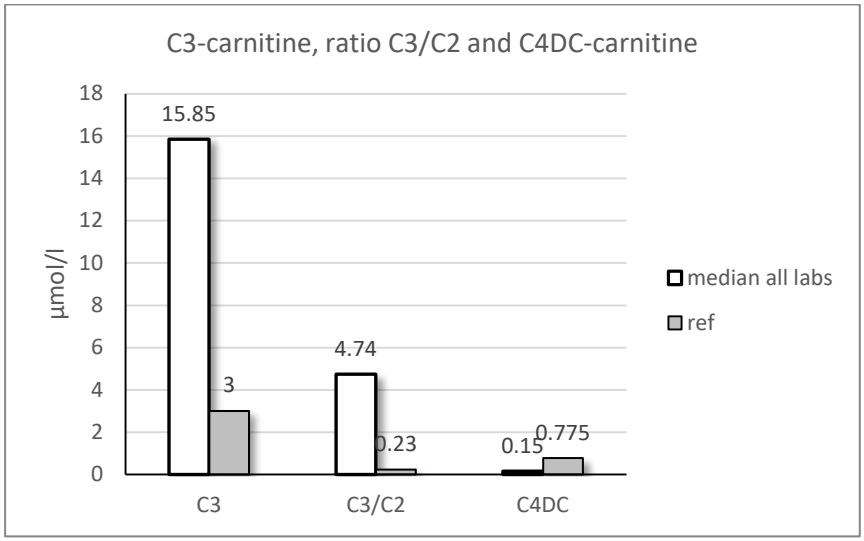
Sample ACDB_DH_2019-A:

Patient details: 7-month-old boy admitted with metabolic acidosis, hyperammonaemia

Known diagnosis: propionyl-CoA carboxylase deficiency (propionic acidaemia)

Analytical details: This sample showed elevated amounts of propionylcarnitine (C3) which was detected by 100% of the participants (41/41). An increased ratio C3/C2 was reported by 46% (19/41).

C4DC-carnitine was normal in this sample.



Interpretation: 90% of the participants diagnosed propionic acidaemia (37/41). Four labs (10%) opted for methylmalonic acidaemia.

Full points were awarded for diagnosing propionic acidaemia or methylmalonic acidaemia as long as organic acids analysis in urine was given as advice for further investigations.

The overall performance was 98%.

Sample ACDB_DH_2019-B:

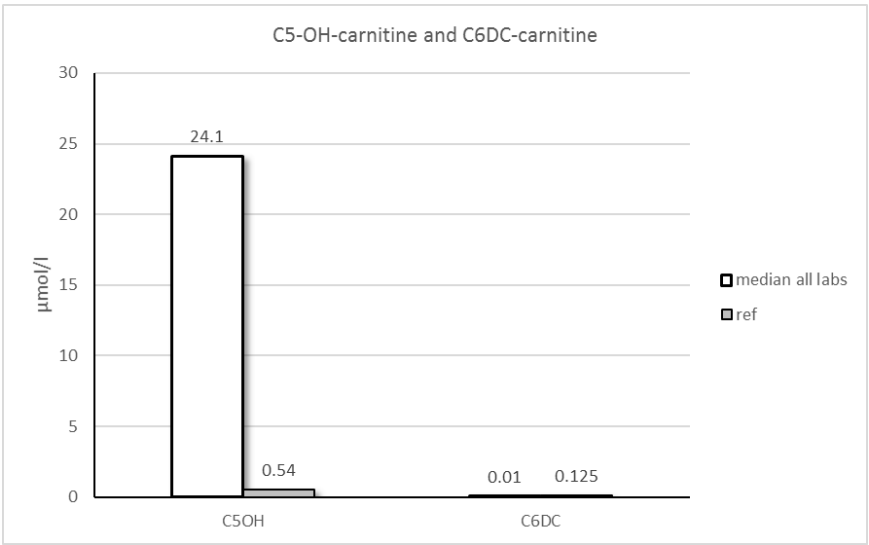
Patient details: 14-year-old male with learning difficulties

Known diagnosis: 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency

Analytical details: Clearly elevated concentrations of C5-OH-carnitine. This was reported by 98% of the participants (40/41).

C6DC-carnitine was normal in this sample which was reported by 27% (11/41).

Analytical performance was 98%.



Interpretation: The correct diagnosis of 3-MCC deficiency was scored with two points. Also two points were given for diagnosing HMG-CoA lyase deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency and 3-methylglutaconyl-CoA hydratase deficiency as long as organic acids analysis in urine was given as advice for further investigations.

The overall performance was 85%.

Sample ACDB_DH_2019-C:

Patient details: 60-year-old male with cognitive decline

Known diagnosis: normal control sample

Analytical details: This was a normal acylcarnitine profile.

Interpretation: Most of the participants found this sample to be normal (35/41).
One point was subtracted for any other diagnosis depending on the recommendations for further investigations.

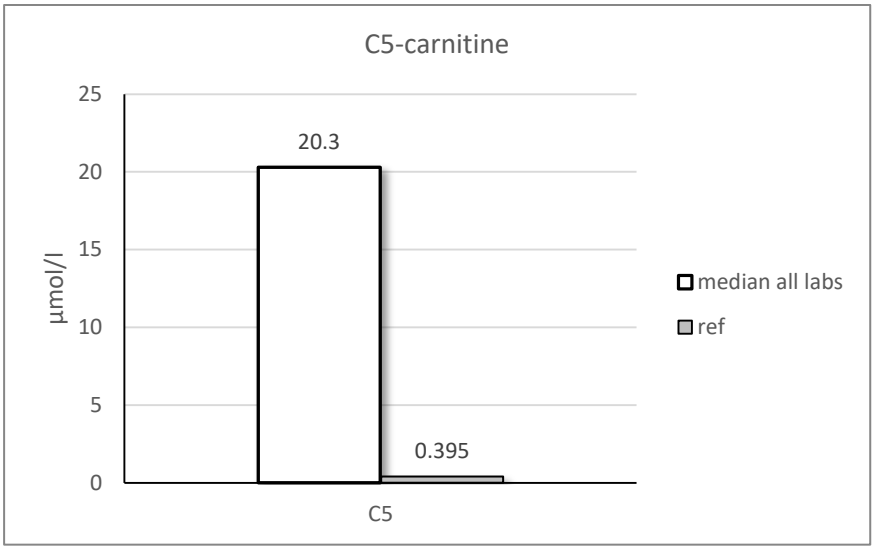
The overall performance was 83%.

Sample ACDB_DH_2019-D:

Patient details: 11-year-old boy, severe metabolic acidosis during gastrointestinal infection

Known diagnosis: isovaleryl-CoA dehydrogenase deficiency (isovaleric acidaemia)

Analytical details: The key finding of elevated C5-carnitine was scored with two points. This was reported by nearly all laboratories (40/41).



Interpretation: Ninety-three labs gave the correct diagnosis of isovalerylCoA dehydrogenase deficiency and received two points for interpretation.

Any other suggested diagnosis such as antibiotic treatment with ketosis or beta-ketothiolase deficiency was scored with one point as long as organic acid analysis in urine was recommended.

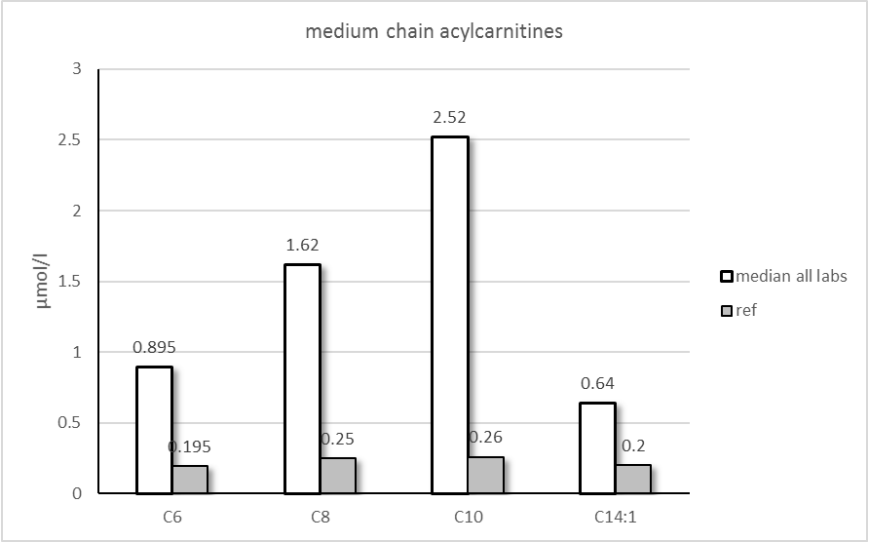
The overall performance was 95%.

Sample ACDB_DH_2019-E:

Patient details: 15-year-old male, episodes of muscle weakness

Known diagnosis: multiple acyl CoA dehydrogenase deficiency (MADD, GA 2)

Analytical details: this sample showed elevated concentrations of several medium chain acylcarnitine species, namely C6-, C8-, C10-, C12-carnitine as well as C14:1-carnitine.



All laboratories reported these metabolites in different combinations.

Analytical performance was very good (100%).

Two points were awarded for reporting at least two of the relevant metabolites.

Interpretation: 71% of the participants (29/41) diagnosed MADD and scored 2 points. MCADD which was diagnosed by 17% (7/41) scored one point. Two points were awarded only when organic acid analysis was suggested as further investigation.

The overall performance was 88%.

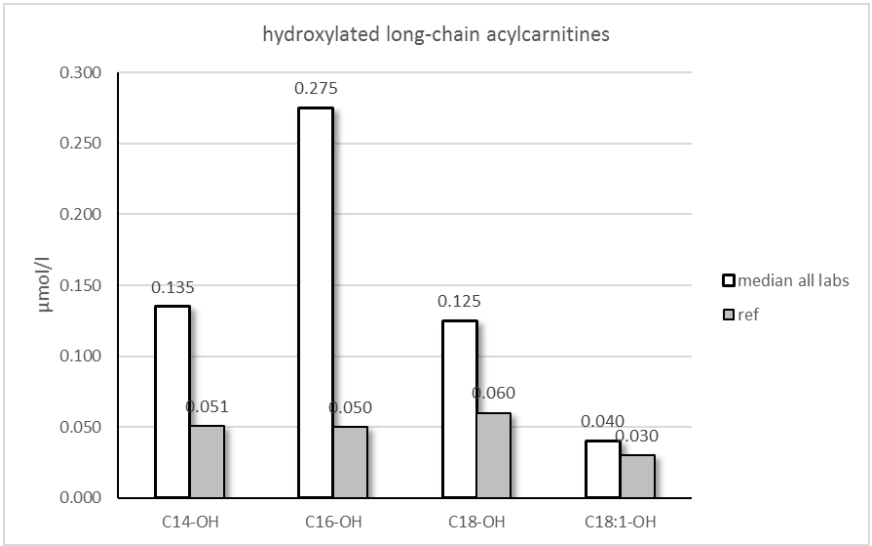
Sample ACDB_DH_2019-F:

Patient details: 13-year-old boy, first referred at the age of 14 days with hypothermia, coma and circulatory failure. Currently under treatment

Known diagnosis: long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

Analytical details: Relevant metabolites in this sample were hydroxylated long-chain acylcarnitines C14-OH-carnitine, C16-OH-carnitine, C18-OH-carnitine, and C18:1-OH-carnitine.

These were reported in different combinations by 88% of the participants.



Interpretation: 88% of the participants diagnosed LCHAD deficiency or mitochondrial trifunctional protein (MTP) deficiency (36/41) and scored two points.

The overall performance was 88%.

In the November meeting the ERNDIM SAB considered the failure to detect elevated concentrations of hydroxylated long-chain acylcarnitines as a critical error. This was applied to three laboratories who gave a normal diagnosis.

10. Scores of participants

Table 8 presents detailed scores and performance data for all participants.

Scores and performance data were confirmed by the Scientific Advisory Board meeting in November 2019.

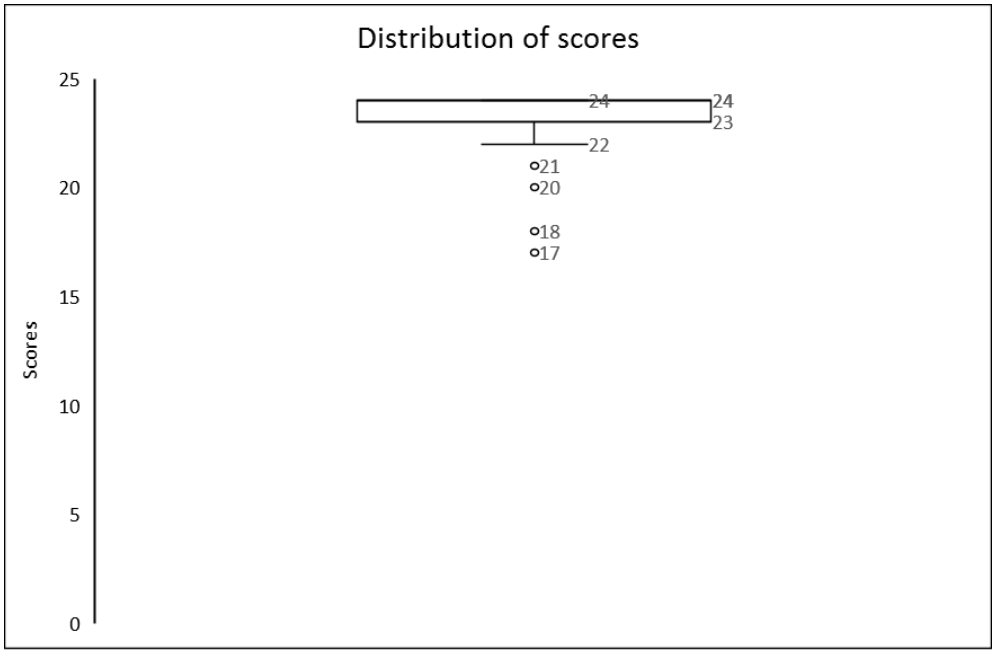
The anonymous data are accessible to all participants. Individual data are only visible to your laboratory

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
1										Educational
2	4	4	4	12	4	4	4	12	24	
3	4	4	4	12	4	4	4	12	24	
4	4	4	3	11	4	4	4	12	23	
5	4	4	4	12	4	3	4	11	23	
6	4	4	4	12	4	4	4	12	24	
7										non-submitter
8	4	4	4	12	4	4	4	12	24	
9	4	4	4	12	4	4	4	12	24	
10	4	4	4	12	4	3	4	11	23	
11	4	4	3	11	3	4	4	11	22	
12	4	2	3	9	4	4	4	12	21	
13	4	4	4	12	4	4	4	12	24	
14	4	4	4	12	4	4	4	12	24	
15	4	4	4	12	4	4	4	12	24	
16	4	1	3	8	4	4	4	12	20	
17	4	4	4	12	4	4	4	12	24	
18	4	4	4	12	4	4	0	8	20	CE
19	4	4	4	12	4	4	4	12	24	
20	4	4	3	11	4	4	1	9	20	
21	4	4	4	12	4	4	4	12	24	
22	4	4	4	12	4	4	4	12	24	
23	4	4	4	12	4	4	4	12	24	
24										non-submitter
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	4	3	3	10	4	4	0	8	18	CE
29	4	4	4	12	4	3	4	11	23	
30	4	4	4	12	4	4	4	12	24	
31	4	3	4	11	4	4	4	12	23	
32	4	4	4	12	4	4	4	12	24	
33	4	4	4	12	4	4	4	12	24	
34	4	4	4	12	4	4	4	12	24	
35	4	3	4	11	2	4	0	6	17	CE
36	4	4	4	12	4	3	4	11	23	
37	4	4	4	12	4	4	4	12	24	
38	4	4	4	12	4	4	4	12	24	
39	4	4	4	12	4	4	4	12	24	
40	4	4	4	12	4	4	4	12	24	

Lab no	A	B	C	sum	D	E	F	sum	Total score	Performance
41	3	4	4	11	4	3	4	11	22	
42	4	4	4	12	4	4	4	12	24	
43	4	4	4	12	4	4	4	12	24	
44										non-submitter

CE: Critical error

Figure 1: Boxplot presentation of all scores



ERNDIM Number:

Lab number in the above table:

Your laboratory scores for 2019:

Maximum total score 2019: 24

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 13, 2020

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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