ERNDIM Quantitative Schemes Acylcarnitines in Serum

ANNUAL REPORT 2023

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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1. Purpose

The purpose of the ERNDIM External Quality Assurance Scheme for Acylcarnitines in Serum is the monitoring of the analytical quality of the quantitative assay of a range of analytes in serum in laboratories involved in the diagnosis of patients with inherited metabolic disorders. For details, see www.erndim.org / www.ERNDIMQA.nl

2. Participants

A total of 126 datasets have been submitted, for 2 of them an annual report could not be generated due to insufficient data submission. 3 laboratories did not submit results at all.

3. Design

The Scheme has been designed, planned and co-ordinated by the scientific advisor Dr. P. Ruiz-Sala and Dr. C.W. Weykamp as scheme organizer (on behalf of MCA Laboratory), both appointed by and according to the procedure of the ERNDIM Board. The design includes samples and reports to provide information with a balance between short-term and long-term reports and between detailed and aggregated information. As a subcontractor of ERNDIM, the MCA Laboratory prepares and dispatches EQA samples to the scheme participants and provide a website for on-line submission of results and access to scheme reports.

Samples

The scheme consisted of 8 lyophilized samples, all prepared from the same basic serum but with various amounts of added analytes. The samples were identical two by two: the pairs, analytes and their source as well as the added amounts are in the table below. Samples have been tested for stability and homogeneity according to ISO 13528.

[1] If this Annual Report is not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document

Joined to the analytes, total carnitine has been added to the list to be measured. Total carnitine is not a spiked analyte.

Table 1.

Analyte	Source:	Added Amounts (µmol/L)			
		Sample Pair 2023. 01 - 07	Sample Pair 2023. 02 - 05	Sample Pair 2023. 03 - 06	Sample Pair 2023. 04 - 08
Free carnitine (C0)	C0283 (Sigma)	0,0	60,0	40,0	10,0
Acetylcarnitine (C2)	10117 (VUMC*)	25,0	15,0	10,0	0,0
Propionylcarnitine (C3)	VUMC*	8,0	2,0	0,0	15,0
Butyrylcarnitine (C4)	10142 (VUMC*)	0,0	5,0	3,0	1,0
3-OH-Butyrylcarnitine (C4-OH)	H830900 (Bio-Connect)	0,0	5,0	2,0	0,3
Tiglylcarnitine (C5:1)	VUMC*	1,0	0,3	0,0	2,0
Isovalerylcarnitine (C5)	VUMC*	1,5	0,0	6,0	3,0
3-OH-Isovalerylcarnitine (C5-OH)	H943620 (TRC)	0,3	0,0	2,5	1,0
Hexanoylcarnitine (C6)	VUMC*	2,0	1,0	0,6	0,0
Octanoylcarnitine (C8)	VUMC*	1,5	0,8	0,0	6,0
Decanoylcarnitine (C10)	VUMC*	0,5	0,0	5,0	2,0
Dodecanoylcarnitine (C12)	VUMC*	0,0	4,0	2,0	0,5
cis-5-Tetradecenoylcarnitine (C14:1)	T291425 (TRC)	2,4	1,2	0,4	0,0
Palmitoylcarnitine (C16)	9330 (VUMC*)	2,4	0,6	0,0	3,6
3-OH-Palmitoylcarnitine (C16-OH)	H943005 (TRC)	0,1	0,0	1,2	0,8
Oleoylcarnitine (C18:1)	O526700 (TRC)	0,0	1,6	0,8	0,2
Stearoylcarnitine (C18)	VUMC*	1,2	0,8	0,4	0,0
3-OH-Stearoylcarnitine (C18-OH)	H953630 (TRC)	0,7	0,1	0,0	1,2
Malonylcarnitine (C3-DC)	M158150 (TRC)	0,1	0,0	1,2	0,7
Methylmalonylcarnitine (C4-DC)	M318900 (TRC)	1,2	0,7	0,1	0,0
Glutarylcarnitine (C5-DC)	G597605 (TRC)	0,0	3,0	1,5	0,5

** Supplied by University of Amsterdam*

Reports

All data-transfer, the submission of data as well as request and viewing of reports proceeded via the interactive website www.erndimqa.nl which can also be reached through the ERNDIM website (www.erndim.org). The results of your laboratory are confidential and only accessible to you (with your name and password). The anonymised mean results of all labs are accessible to all participants. Statistics of the respective reports are explained in the general information section of the website.

An important characteristic of the website is that it supplies short-term and long-term reports. Short-term reports are associated with the four individual specimens, for each of which there has been a specific deadline in the year 2023. Two weeks after the respective deadlines participants could request their reports and as such had four times up-to-date information on their analytical performance. Although technically not required (the website can work without any delay time) a delay time of 14 days has been chosen to enable the scientific advisor to inspect the results and add his comment to the report. Contrary to the fast short-term report is the annual long-term report. The annual report is based on the design-anchored connection between samples which enables to report a range of analytical parameters (accuracy, precision, linearity, recovery and interlab dispersion) once an annual cycle has been completed. The annual report is discussed below.

A second important characteristic of the website is the wide range in aggregation of results which permits labs to make an individual choice for detailed and/or aggregated reports. The most detailed report which can be requested from the website is the

"Analyte in Detail" which shows results of a specific analyte in a specific sample (168 such Analyte-in-Detail-reports can be requested in the 2023 cycle). A more condensed report is the "Cycle Review" which summarizes the performance of all analytes in a specific sample (8 such Cycle-Review-Reports can be requested in 2023). The highest degree of aggregation has the Annual Report which summarizes the performance of all analytes of all 8 samples (1 such Annual-Report can be requested in 2023).

4. Discussion of Results in the Annual Report 2023

In this part the results as seen in the annual report 2023 will be discussed. Subsequently we will regard accuracy, recovery, precision, linearity, interlab CV and cross sectional relations. Please keep at hand your annual report from the Interactive Website when you read the "guided tour" below and keep in mind that we only discuss the results of "all labs": it is up to you to inspect and interpret the specific results of your laboratory.

4.1 Accuracy

A first approach to describe the accuracy is to compare mean outcome in your lab of the eight samples with the mean outcome of all labs. This is done in the first columns of the annual report. It can be seen that the mean outcome for all labs for free carnitine (C0) is 62.6 µmol/L.

4.2 Recovery

A second approach to describe accuracy is the percentage recovery of added analyte. In this approach it is assumed that the recovery of the weighed quantities is the target value. The correlation between weighed quantities as added to the samples (on the x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the correlation multiplied by 100% is your recovery of the added amounts. Outcome for your lab in comparison to median outcome of all labs is shown in the column "Recovery" in the Annual Report. For all labs the recovery ranges from 54% for 3-OH-butyrylcarnitine (C4-OH) to 131% for dodecanoylcarnitine (C12).

4.3 Precision

Reproducibility is an important parameter for quality in the laboratory and is encountered in the schemes' design. Samples come in pairs which can be regarded as duplicates from which CV's can be calculated (Intra laboratory CV as indicator for reproducibility). Outcome for your lab in comparison to the median of all labs is shown in column "Precision" of the Annual Report. Precision ranges from 8.4% for free carnitine (C0) to 18.8% for malonylcarnitine (C3-DC). The overall precision of 11.7% is satisfying.

4.4 Linearity

Linearity over the whole relevant analytical range is another important parameter for analytical quality. Again this is encountered in the Schemes' design. With weighed quantities on the x-axis and your measured quantities on the y-axis the coefficient of regression (r) has been calculated. Outcome for your lab in comparison to the median of all labs is in the column "Linearity" of the annual report. It can be seen that the coefficient of regression is best for octanoylcarnitine (C8) (0.997) and lowest for acetylcarnitine (C2) (0.980).

4.5 Interlab CV

For comparison of outcome for one patient in different hospitals and for use of shared reference values it is relevant to have a high degree of harmonization between results of various laboratories. Part of the schemes' design is to monitor this by calculating the

Interlaboratory CV. This, along with the number of laboratories who submitted results, is shown in the column “Data all Labs” in the Annual Report. It can be seen that most laboratories submitted results for free carnitine, propionylcarnitine (C3) and palmitoylcarnitine (C16) (122) whereas 88 labs submitted results for methylmalonylcarnitine (C4-DC). The Interlab CV ranges from 13.1% for free carnitine (C0) to 48.5% for methylmalonylcarnitine (C4-DC).

4.6 Cross Sectional Relations

The various parameters as described above often have an interrelation: more than one parameter directs towards good or bad analytical control.

4.7 Your performance: Flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags in case of poor performance for accuracy, precision, linearity and recovery. Analytes with satisfactory performance for at least three of the four parameters (thus no or only one flag) receive a green flag. Thus a green flag indicates satisfactory performance for analysis of that particular analyte. Criteria for flags can be found in the general information on the website (on this website under general information; interactive website, explanation annual report).

4.8 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of flags observed. 30% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 7% of laboratories with more than 25% red flags. Following intensive discussion within the ERNDIM board and Scientific Advisory Board (SAB) and taking into account feedback from participants we have been able to agree on a harmonised scoring system for the various branches of the Diagnostic Proficiency schemes and qualitative schemes. We have also tested a scoring system for the quantitative schemes as described in our Newsletter of Spring 2009. In parallel to this, the SAB has agreed levels of adequate performance for all the schemes and these will be re-evaluated annually. The scoring systems have been carefully evaluated by members of the SAB and have been applied to assess performance in our schemes from 2007 onwards. The ERNDIM Board has decided that the Scientific Advisor will judge the performance of the individual laboratories based on these levels of satisfactory performance and issue a letter of advice of failure to achieve satisfactory performance to those laboratories which do not achieve satisfactory performance. The letter is intended to instigate dialogue between the EQA scheme organiser and the participating laboratory in order to solve any particular analytical problems in order to improve quality of performance of labs in the pursuit of our overall aim to improve quality of diagnostic services in this field.

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. Details of how to appeal poor performance are included in the Performance Support Letter sent to poor performing laboratories.

Table 2. Percentage Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	3%	3%
25%	1%	4%
20 – 25%	1%	5%
15 – 20%	6%	11%
10 – 15%	10%	21%
5 – 10%	15%	36%
0 – 5%	39%	75%
0%	25%	100%

4.9 Certificates

As for other schemes the performance as it is indicated by the red/green flags in the individual laboratories annual report is summarised in the new style of annual participation certificate. The certificate lists the total number of special assays in the scheme, the number for which results have been submitted and the number for which satisfactory performance has been achieved. It is important to bear in mind that the certificate has to be backed up by the individual annual report in the case of internal or external auditing.

4.10 Additional Specific Remarks of the Scientific Advisor

In general, participants who have checked the options of methods that use calibration curves (usually, while not derivatizing and acquiring by MRM) obtain good results, which is expected. This does not mean that participants who use derivatization and precursor ion acquisition will provide worse results, but it is true that some participants increase the dispersion of these results.

It is observed that few participants mark the option of using certain deuterated standards, such as D9-C5 for C5:1 or D3-C6 for C6. This contradicts the result of the survey that was previously sent to the participants, in which these deuterates standards were chosen in the majority.

The low participation of laboratories that use methods without derivatization for the quantification of C3-DC, C4-DC, C4-OH and C5-OH persists, to avoid bad results in the scheme, because they possibly know that the use of these methods does not prevent giving good results in the analysis of real samples, both in healthy controls and in patients in whom these biomarkers are elevated as a consequence of a certain metabolic defect.

5. Summary

The Annual Report deals with analytical performance in terms of accuracy, precision, linearity, recovery and interlab CV. All parameters (intralab CV, linearity, recovery, interlab CV and number of partcipating laboratories) demonstrate slightly better performance when compared to 2022.

6. Preview Scheme 2024

The design of the 2024 scheme is similar to the 2023. We hope that the results obtained in 2023 after the changes in the choice of methods will help the participants in 2024 to improve their results or confirm their good results

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the scientific advisor of the scheme Dr. P. Ruiz-Sala (prsala@cbm.csic.es) and/or to the scheme organiser Dr. C.W. Weykamp (mca.office@skbwinterswijk.nl)

Madrid, 9th January 2024

Dr. P. Ruiz-Sala
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Acylcarnitines in Serum scheme. The contents should not be used for any publication without permission of the scheme advisor.

The fact that your laboratory participates in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will be shared within ERNDIM for the purpose of evaluating your laboratory performance, unless ERNDIM is required to disclose performance data by a relevant government agency. For details, please see the terms and conditions in the ERNDIM Privacy Policy on www.erndim.org.

APPENDIX 1. Change log (changes since the last version)

Version Number	Published	Amendments
1	9th January 2024	• 2023 annual report published

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