ERNDIM
Quantitative Schemes
Acylcarnitines in Serum

ANNUAL REPORT 2018

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
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Madrid-Winterswijk, 17 December 2018

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 99 datasets have been submitted, for 3 of them an annual report could not be generated due to insufficient data submission. 5 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. Cas Weykamp as scheme organizer (subcontractor on behalf of SKML), both appointed by and according to the procedure of the ERNDIM Board. The design includes samples and reports which are connected to provide information with a balance between short-term and long-term reports and between detailed and aggregated information.

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.

This year, joined to the analytes, total carnitine has been added to the list to be measured, in response to the suggestions of the participants. Total carnitine is not a spiked analyte.

Table 1.

Analyte	Source:	Added Amounts (µmol/L)			
		Sample Pair 2018. 01 - 05	Sample Pair 2018. 02 - 07	Sample Pair 2018. 03 - 08	Sample Pair 2018. 04 – 06
Free carnitine	C0283 (Sigma)	0	40	10	60
Acetylcarnitine	10117 (Vumc)	0	25	15	10
Propionylcarnitine	Vumc*	0	2,0	15	8,0
Butyrylcarnitine	10142 (Vumc)	0	5,0	3,0	1,0
Tiglylcarnitine	Vumc*	0	1,0	0,3	2,0
Isovalerylcarnitine	Vumc*	0	6,0	3,0	1,5
Hexanoylcarnitine	Vumc*	0	1,0	0,6	2,0
Octanoylcarnitine	Vumc*	0	6,0	1,5	0,8
Decanoylcarnitine	Vumc*	0	0,5	5,0	2,0
cis-5-Tetradecenoylcarnitine	Brunet	0	1,2	0,4	2,4
Tetradecanoylcarnitine	Vumc*	0	1,2	0,8	0,2
Palmitoylcarnitine	9330 (Vumc)	0	0,6	3,6	2,4
3-OH Palmitoylcarnitine	Brunet	0	0,8	0,1	1,2
Oleoylcarnitine	O526700 (TRC)	0	1,6	0,8	0,2
Stearoylcarnitine	Vumc*	0	0,4	1,2	0,8
3-OH stearoylcarnitine	Brunet	0	0,7	0,1	1,2
Malonyl carnitine	M158150 (TRC)	0	0,7	0,1	1,2
Methyl malonylcarnitine	M318900 (TRC)	0	0,1	1,2	0,7
Glutarylcarnitine	G597600 (TRC)	0	0,5	3,0	1,5

** 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 2017. 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 with a delay time zero) 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 (160 such Analyte-in-Detail-reports can be requested in the 2018 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 2018). 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 2018).

4. Discussion of Results in the Annual Report 2018

In this part the results as seen in the annual report 2018 will be discussed. Subsequently we will regard accuracy, recovery, precision, linearity, interlab CV and cross sectional relations. Please print 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 Carnitine Free is 64.5 micromol/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 with 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 5% for cis-5-Tetradecenoylcarnitine (may be due to the low purity of the standard) to 96% for Tetradecanoylcarnitine and Palmitoylcarnitine.

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 8.0% for Carnitine Free to 21.5% for Methylmalonylcarnitine. The overall precision of 13.3% 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 (0.997) and lowest for cis-5-Tetradecenoylcarnitine (0.927).

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 Propionylcarnitine (99) whereas 74 labs submitted results for 3-OH-Sterarocylcarnitine. The Interlab CV ranges from 15.4% for Carnitine Free to 77% for Methylmalonylcarnitine.

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. 28% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 5% 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.

Table 2. Percentage Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	5%	5%
25%	0%	5%
20 – 25%	0%	5%
15 – 20%	6%	11%
10 – 15%	14%	25%
5 – 10%	13%	38%
0 – 5%	34%	72%
0%	28%	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 order to improve the list of methods that the participants could choose (to be more representative of the different practices and also give more information that could explain better the results), a survey was circulated previously of the beginning of the scheme. The answers of the participants allowed changing these lists of methods. However, the election of the method in the scheme has not been the expected based on the answers of the survey. For example, 45/98 participants in the survey answered they used deuterated-C5DC; but only 14/85 have indicated this option in during the scheme.

Other conclusion to consider is that after inclusion of Total Carnitine in the scheme, as many participants requested, the methods used were mass spectrometry based (45/48), without any result made by colorimetric nor radiochemical methods, conversely to the analysis of Free Carnitine in the Special Assays schemes. This fact could be due to the decision of these laboratories to not participate in the ACS scheme.

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 partcipicating laboratories) demonstrate slightly better performance when compared to 2017.

6. Preview Scheme 2019

The design of the 2019 scheme is essentially the same as in 2018, new analytes are 3-Hydroxybutyrylcarnitine (C4-OH) and 3-Hydroxyisovaleroylcarnitine (C5-OH)

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. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)

Madrid, 17 December 2018

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.