ERNDiM
Quality Assurance in Laboratory Testing for IEM

ERNDIM Quantitative Schemes Special Assays in Serum

ANNUAL REPORT 2021

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 Special Assays 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 278 datasets (227 labs) have been submitted, for 8 of them an annual report could not be generated due to insufficient data submission. 2 laboratories did not submit results at all.

3. *Design*

The Scheme has been designed, planned and co-ordinated by the scientific advisor (Mr. Rafael Artuch) and Dr. Eline van der Hagen 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, except for biotinidase activity, that

[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

it was that present as endogenous activity in the lyophilized samples and it was not added (results are the mean values reported by the different laboratories). 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.

Table 1.

Analyte	Source:	Units	Added Amounts			
			Sample Pair 2021. 01 - 05	Sample Pair 2021. 02 - 07	Sample Pair 2021. 03 - 06	Sample Pair 2021. 04 – 08
3-OH-Butyric Acid	Aldrich 29.836-0	mmol/L	0.00	0.90	2.35	3.79
7-Dehydrocholesterol	Sigma 30800	µmol/L	0.00	163	71.4	8.74
7-Ketocholesterol	Sigma C2394	µmol/L	0.09	1.45	0.48	0.96
Biotinidase	Endogenous	nmol/min/ml serum	5.5	5.3	4.7	5.5
C22:0	Aldrich 21.694-1	µmol/L	0.00	15.4	53.9	34.7
C24:0	Sigma L6641	µmol/L	0.93	29.9	44.3	10.6
C26:0	Sigma H0388	µmol/L	0.00	8.65	0.95	3.89
Carnitine Free	Sigma C0283	µmol/L	0.00	11.6	59.9	98.2
Cholestane-3b. 5a. 6b-triol	Biozol T795100	µmol/L	0.05	0.48	0.73	0.24
Cholestanol	Sigma D6128	µmol/L	0.00	89.5	12.5	65.5
Coenzyme Q10	Sigma C9538	µmol/L	0.00	1.91	3.85	0.95
Creatine	Sigma C3630	µmol/L	0.00	22.1	41.4	60.7
Galactose	Sigma G0750	µmol/L	48.2	1829	1155	481
Glucosylsphingosine	Sigma 43659	nmol/L	0.00	386	580	966
Guanidine acetate	Aldrich G11608	µmol/L	0.94	5.86	12.6	18.2
Homocystine	Sigma H6010	µmol/L	0.00	38.6	192.6	14.7
Lactic Acid	Sigma L7022	mmol/L	0.00	6.2	2.1	4.0
LysoGb3	Sigma G9534	nmol/L	0.00	48.0	96.5	9.6
Lysoshpingomyelin	Cayman Chem. 100079475	nmol/L	0.00	7.4	48.0	3.7
Methylmalonic acid	Aldrich M5.405-8	µmol/L	0.00	48.2	385	2.03
Phytanic acid	Sigma P4060	µmol/L	0.00	21.2	6.70	14.5
Pipecolic Acid	Sigma P2519	µmol/L	0.00	7.97	19.4	36.6
Pristanic acid	Sigma P6617	µmol/L	0.00	4.83	6.78	1.95
Pyruvic Acid	Sigma B8574	mmol/L	0.05	0.14	0.19	0.10

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 2021. 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 (208 such Analyte-in-Detail-reports can be requested in the 2021 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 2021). 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 2021).

4. Discussion of Results in the Annual Report 2021

In this part the results as seen in the annual report 2021 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.

Biotinidase. Cholesterol and NEFA are not included in the annual report because these analytes have not been spiked which makes it impossible to calculate recovery and linearity.

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 free is 69.1 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 26% for Coenzyme Q10 to 110% for Lyso Gb3.

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 4.1% for lactic acid to 50.5% for Coenzyme Q10. The overall precision of 11.1% is quite 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 Homocysteine and Methylmalonic acid (0.999) and lowest for Coenzyme Q10 (0.664).

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 3 OH Butyric acid (n=129) whereas only 10 labs submitted results for 7-Ketocholesterol. The Interlab CV ranges from 5.18% for Lactic Acid to 82.2% for Coenzyme Q10.

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.
A typical example of good analytical control is lactic acid: many (88) laboratories submitted results, the reproducibility within the labs is good (precision of 4.1%), the interlab CV is good (5.18%), linearity is good (0.996) as is the recovery (100%).

4.7 Your performance: Flags

In order to easily judge performance of individual laboratories the annual report of an individual laboratory may include flags with different colours 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. 58% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme there are also 6% 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%	6%	6%
25%	5%	11%
20 – 25%	1%	12%
15 – 20%	3%	15%
10 – 15%	8%	23%
5 – 10%	10%	33%
0 – 5%	9%	42%
0%	58%	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 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

The Annual Report. dealing with analytical performance in terms of accuracy. precision. linearity. recovery and interlab CV. shows a good overall performance. The less satisfying results assessed as precision (CV% of duplicates and interlab CV) affects CoQ10, lysosphingomyelin, lyso GB3 and 7-ketocholesterol, all of them showing CV % of the duplicates higher than 15%). A plausible explanation can be that the number of Labs reporting results is low (from 10 to 26).

Concerning the newly added in 2020 CoQ10, no good results were observed, displaying again a very high CV%, poor precision and recovery. However, the interlab CV value significantly decreased from 2020 to 2021. Either stability troubles or the low number of participants (n=13) would explain these results. We will analyze the trend of metrological parameters in 2022 to take a decision about to continue with CoQ in the SAS scheme.

5. Summary

The Annual Report. dealing with analytical performance in terms of accuracy. precision. linearity. recovery and interlab CV. shows a performance with similarities to previous years. For some analytes the performance is good. for others there is still something to do to achieve sufficient intra- and interlaboratory quality. In comparison to the previous scheme performance is very similar.

The poor performance observed for CoQ will be analyzed in future internal meeting in order to asses the causes and its inclusion in future schemes. As commented, some degree of improvement were observed this year when compared to 2020 performance

6. Preview Scheme 2022

The design of the 2022 scheme is essentially the same as in 2021. Succinylacetone will be included in 2022.

7. Questions. Comments and Suggestions

If you have any questions. comments or suggestions please address to the scientific advisor of the scheme Mr. Rafael Artuch (Rartuch@sjdhospitalbarcelona.org) and/or to the scheme organiser Dr. Eline van der Hagen(E.vanderHagen@skbwinterswijk.nl)

Barcelona. 15 April 2022

Mr. Rafael Artuch
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Special Assays 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	14 December 2021	• 2021 annual report published
2	15 April 2022	• New ERNDIM logo, change of Administration Office address (heading) and change text footnote[1]

END