ERNDIM Quantitative Schemes Special Assays in dried blood spots

ANNUAL REPORT 2019

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 dried blood spots is the monitoring of the analytical quality of the quantitative assay of a range of analytes in dried blood spots 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 87 datasets have been submitted, for 11 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 advisors Prof. Jim Bonham and Dr. Rachel Carling and Dr. Cas Weykamp as scheme organizer (subcontractor on behalf of SKML), all three 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. As a subcontractor of ERNDIM, SKML prepare and dispatch 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 10 dried blood spots, five identical pairs, all prepared from the same basic whole blood but with various amounts of added analytes. Blood was depleted to create low concentrations but not to a zero level. Thus concentrations of the samples is the remaining physiological concentration plus the spiked amount. The

analytes and their source, as well as the added amounts, are shown in the table below for each pair. Samples have been tested for stability and homogeneity according to ISO 13528.

Table 1.

Analyte	Source:	Added Amounts (µmol/L)				
		Sample Pair 2019. 01 - 10	**Sample Pair 2019. 02 - 06**	**Sample Pair 2019. 03 - 08**	**Sample Pair 2019. 04 – 07**	**Sample Pair 2019. 05 – 09**
Allo-isoleucine	Sigma I8454	0,0	5,0	19,8	49,9	99,9
Free Carnitine	Sigma C0283	8,0	18,0	28,0	47,9	3,0
Total Homocysteine	Sigma H6010	95,9	1,0	16,0	26,0	46,0
Isoleucine	Roth 3922.1	184,8	434,4	784,9	34,3	64,8
Leucine	Roth 3984.1	651,8	1452,2	53,4	102,9	301,1
Methionine	Sigma 64319	0,0	10,1	39,9	190,0	390,1
NTBC	Sigma PHR1731	5,0	15,0	30,0	60,0	0,0
Phenylalanine	Sigma 78019	275,4	375,3	974,6	27,2	57,5
Succinylacetone	Sigma D1415	5,0	20,0	0,0	0,5	2,0
Tyrosine	Sigma 93829	880,3	30,4	58,0	278,7	579,5
Valine	Roth 4879.1	0,0	25,6	145,1	396,9	644,5

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 on the ten individual specimens are available two weeks after the submission deadline and provide up-to-date information on analytical performance. Although it is technically possible to produce reports immediately there is a delay of 14 days to enable the scientific advisor to inspect the results and add comments to the report when appropriate.

The ***annual long-term 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 (110 such Analyte-in-Detail-reports can be requested in the 2019 cycle). A more condensed report is the “Cycle Review” which summarizes the performance of all analytes in a specific sample (10 such Cycle-Review-Reports can be requested in 2019). The highest degree of aggregation has the Annual Report which summarizes the performance of all analytes of all 10 samples (1 such Annual-Report can be requested in 2019).

4. Discussion of Results in the Annual Report 2019

In this part the results as seen in the annual report 2019 will be discussed. Subsequently we will regard accuracy, recovery, precision, linearity, interlab CV and relations between these parameters. 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 the mean outcome of the ten samples from your lab with the mean outcome from 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 alloisoleucine is 34.8 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 33% for succinylacetone to 136% for total homocysteine.

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. The precision ranges from 6.5% for NBTC to 18.6% for succinylacetone.

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 for phenylalanine is0.99)

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 phenylalanine (78) whereas only 9 labs submitted results for NBTC. The Interlab CV ranges from 20.3% for tyrosine to 112% for succinylacetone.

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. 53% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme, 4% of laboratories have 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%	4%	4%
25%	5%	9%
20 – 25%	0%	9%
15 – 20%	1%	10%
10 – 15%	13%	23%
5 – 10%	12%	35%
0 – 5%	12%	47%
0%	53%	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

N/A

5. Summary

The Annual Report deals with analytical performance in terms of accuracy, precision, linearity, recovery and inter-lab CV. All parameters (intra-lab CV, linearity, recovery, inter-lab CV and the number of participating laboratories) demonstrate slightly better performance when compared to 2019 for those analytes that were also included last year.

6. Preview Scheme 2020

We have modified the design of the scheme for 2020. In the 2018 scheme we distributed aa calibrator blood spot for phenylalanine. Results showed that virtual calibration against this calibrator significantly reduced the interlaboratory CV, from 20 to 10%, demonstrating that calibration bias is a significant contributor to interlaboratory variation. This has also been described in a paper (Moat SJ, Schulenburg-Brand D, Lemonde H, Bonham JR, Weykamp CW, Mei JV, Shortland GS, Carling RS. Performance of laboratory tests used to measure blood phenylalanine for the monitoring of patients with phenylketonuria.)
In the 2020 scheme we will again distributecalibrator spots. The annual cycle will consist of 4 filter papers, each containing 2 EQA blood spot samples and 1 calibrator spot. Details will be in the package insert that comes along with the samples (February 2020).
In 2020 Dr. Rachel Carling will take over the task of Scientific Advisor of Prof. Jim Bonham and Prof. Stuart Moat will become the Deputy Advisor.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions please address to the scientific advisor of the scheme Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) and/or to the scheme organiser Dr. Cas Weykamp (c.w.weykamp@skbwinterswijk.nl)

London, 03/02/20

Dr. Rachel Carling
Scientific Advisor