ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2023

Scheme Organiser	Scientific Advisor	Website for reporting results	Administration office
Dr. C.W. Weykamp Streekziekenhuis Koningin Beatrix MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail: mca.office@skbwinterswijk.nl	Dr. Rachel Carling Biochemical Sciences, Viapath 4th Floor, North Wing St Thomas' Hospital London SE1 7EH United Kingdom e-mail: Rachel.Carling@viapath.co.uk Prof. B. Fowler University Children's Hospital Basel CH – 4031 Basel Switzerland e-mail : brian.fowler@unibas.ch	Mrs. Irene de Graaf Streekziekenhuis Koningin Beatrix MCA Laboratory Beatrixpark 1 7101 BN Winterswijk The Netherlands e-mail : i.degraaf@skbwinterswijk.nl	ERNDIM Administration Office c/o EMQN CIC, Unit 4, Enterprise House, Manchester Science Park Pencroft Way Manchester M15 6SE United Kingdom. e-mail: admin@erndim.org

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1. *Purpose*

The purpose of the ERNDIM External Quality Assurance Scheme for Quantitative Amino Acids is the monitoring of the analytical quality of the quantitative assay of amino acids in plasma in laboratories involved in the screening and diagnosis of patients with inherited metabolic disorders. For details see www.erndim.org / www.ERNDIMQA.nl

2. *Participants*

A total of 295 datasets have been submitted. Due to insufficient data submission, it has not been possible to generate annual reports for 11 of them. Six laboratories did not submit any results.

3. *Design*

The scheme has been designed, planned and co-ordinated by Dr. Rachel Carling and Prof. Brian Fowler as scientific advisors and Dr. C.W. Weykamp as scheme organiser (on behalf of the MCA Laboratory), each appointed by and according to procedures laid down by the ERNDIM Board. The design includes special attention to sample content and to the layout of reports. Samples are produced with amino acids at concentration ranges seen in healthy controls and/or patients with inborn errors of metabolism although the patterns of amino acid levels may not reflect those in real life. Low levels of amino acids are sometimes included to mimic those seen in treated patients. As a sub-contractor 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.

[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

Samples

The scheme consisted of 8 lyophilised samples, all prepared from the same basic human serum which has been treated to remove most of the amino acids present and to which various amounts of analytes are added. As can be seen from table 1 the added quantities were identical in pairs of the samples. The nature, source and the added amounts of the analytes are also summarised in table 1.

Table 1. Pair identification, source and amounts of added analytes.

		Added quantities (micromol/L)			
Analyte	**Source**	**Sample pair 2023. 01-07**	**Sample pair 2023. 02-05**	**Sample pair 2023. 03-08**	**Sample pair 2023. 04-06**
2-aminobutyric acid	Sigma A1879	45,0	15,5	60,5	29,6
Alanine	Sigma 44526	599,4	109,1	1001,8	309,5
Alloisoleucine	Sigma I8754	39,8	4,4	80,8	23,2
Arginine	Sigma 90538	400,9	10,3	799,7	100,6
Arginino succinic acid	Sigma A5707	50,4	10,4	99,9	25,6
Asparagine	Sigma 51363	75,8	25,6	101,1	51,0
Aspartic acid	Sigma 51572	75,2	25,0	100,3	50,2
Citrulline	USP 046D0	9,9	1749,6	49,7	874,8
Cystine	Sigma 49603	50,1	100,3	24,8	74,9
Glutamic acid	Sigma 95436	201,3	50,5	399,6	99,8
Glutamine	Sigma 76523	1200,0	100,2	1999,7	299,9
Glycine	Sigma 76524	601,0	102,4	999,2	301,5
Histidine	Sigma 73767	74,8	25,2	101,0	49,6
Hydroxyproline	Sigma PHR1939	29,9	10,0	41,0	19,9
Isoleucine	Sigma 56241	99,6	1750,1	49,8	876,1
Leucine	Sigma 76526	359,5	85,2	720,2	119,5
Lysine	Sigma 67448	124,8	500,7	62,8	251,2
Methionine	Sigma 39496	124,5	501,0	31,1	250,0
Ornithine	Sigma O2375	600,9	199,7	799,7	400,3
Phenylalanine	Sigma 40541	600,1	30,4	1801,0	299,5
Proline	Sigma 93693	360,6	30,1	721,3	121,2
Sarcosine	Sigma S7672	125,4	24,1	250,9	52,0
Serine	Sigma 54763	125,7	24,9	248,6	50,1
Sulphocysteine	Abcam Ab146303	40,0	10,1	80,1	19,7
Taurine	Sigma 93019	150,7	50,2	200,5	98,9
Threonine	Sigma 61506	400,9	50,1	400,9	200,0
Thryptophan	Sigma 51145	80,3	19,9	100,2	39,9
Tyrosine	Sigma 91515	600,7	29,6	134,6	300,0
Valine	Sigma 50848	239,0	60,6	361,6	120,4

All amino acids used are of the highest purity that is commercially available. Concentrations < 100 micromol/L are given to one decimal place; Samples have been tested for stability and homogeneity according to ISO 13528 in which requirements for regulatory purposes of quality management systems for medical devices are described.

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 username and password). The anonymised mean results of all laboratories 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 eight 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 report*** summarises the results for the whole year.

A second important characteristic of the website is the different levels of detail of results which allows individual laboratories the choice of fully detailed and/or summarised reports. The “Analyte in Detail” is the most detailed report and shows results of a specific analyte in a specific sample. Thus, for the 29 amino acids in the 2023 cycle, 8 x 29 = 232 such Analyte-in-Detail-reports can be requested. A more condensed report is the “Cycle Review” which summarises the performance of all analytes in a specific sample (eight such Cycle Reviews can be requested in 2023). The Annual Report summarizes all results giving an indication of overall performance for all analytes in all eight samples.
Depending on the responsibilities within the laboratory, participants can choose to review the annual report (e.g. Quality Managers) or the 232 detailed reports (e.g. scientific staff).

Analyte	Accuracy (mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (%added analyte)		Data all labs	
	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	Your Lab	All labs	n	Interlab cv
2-Aminobutyric acid		46.6		7.3%		0.844		65%	203	11.0%
Alanine	474	505	1.9%	4.8%	0.999	0.998	94%	99%	286	8.56%
Alloisoleucine		36.9		9.1%		0.991		96%	219	12.8%
Arginine	311	325	19.8%	6.0%	0.987	0.998	95%	97%	287	9.29%
Argininosuccinic acid		39.0		12.8%		0.990		99%	149	28.3%
Asparagine	58.0	66.5	6.5%	7.3%	0.995	0.989	91%	105%	258	15.4%
Aspartic Acid	69.5	61.2	3.9%	6.2%	0.995	0.991	105%	93%	268	14.7%
Citrulline	652	656	6.3%	6.3%	0.999	0.998	97%	97%	282	15.3%
Cystine		48.1		9.2%		0.977		68%	255	12.6%
Glutamic acid	212	198	4.7%	6.4%	0.999	0.997	111%	102%	283	10.2%
Glutamine	957	887	9.2%	7.2%	0.997	0.998	106%	98%	283	12.2%
Glycine	511	497	4.2%	5.1%	0.998	0.998	104%	98%	285	8.16%
Histidine	66.0	64.6	4.6%	6.4%	0.997	0.987	100%	98%	279	9.44%
Hydroxyproline	27.7	29.4	1.6%	14.3%	0.775	0.775	41%	54%	231	17.0%
Isoleucine	OR	664	OR	5.6%	OR	0.999	OR	95%	292	12.2%
Leucine	OR	312	OR	5.4%	OR	0.996	OR	100%	292	8.64%
Lysine	227	239	6.6%	5.2%	0.997	0.998	103%	99%	287	7.96%
Methionine	231	223	3.0%	4.7%	0.997	0.999	99%	97%	288	8.41%
Ornithine	537	495	8.3%	5.5%	0.984	0.994	103%	97%	287	7.48%
Phenylalanine	636	651	1.3%	5.3%	0.999	0.999	89%	94%	295	11.1%
Proline	259	304	5.3%	6.3%	1.000	0.998	83%	98%	272	9.77%
Sarcosine	110	113	4.8%	8.8%	0.998	0.996	94%	101%	151	13.4%
Serine	113	114	6.7%	5.0%	0.999	0.998	100%	99%	285	8.56%
Sulfocysteine		31.4		12.9%		0.986		82%	100	22.2%
Taurine	120	128	2.4%	5.9%	0.998	0.994	91%	101%	268	8.49%
Threonine	249	264	1.9%	5.0%	0.999	0.997	93%	100%	283	7.32%
Tryptophan	87.0	85.8	8.0%	7.5%	0.987	0.981	96%	90%	238	13.5%
Tyrosine	263	260	2.2%	5.0%	0.998	0.999	93%	96%	296	7.85%
Valine	202	201	7.4%	4.8%	0.996	0.997	98%	99%	294	7.32%
Overall	290	260	5.5%	6.9%	0.986	0.981	95%	94%	258	11.7%

Above is an example of an annual report.

As agreed in 2016, the flagging system has been changed. The explanation of the flags can be found in the General information section (Use Website / Explanation Annual Report)

4. Discussion of Results in the Annual Report 2023

In this part the results as seen in the annual report 2023 will be discussed. Please keep at hand your annual report from the website when you follow the various aspects below and keep in mind that we only discuss the results of “all labs”. It is your responsibility to inspect and interpret the results of your own laboratory.

4.1 Accuracy

A first approach to evaluating your performance in terms of accuracy is comparison of your mean values for each amino acid in the eight samples with those of all laboratories. This is shown in the columns "Your Lab" and "All Labs" under the heading "Accuracy". For example, for alanine, the mean for all laboratories is 505 micromol/litre, with which you can compare the mean of your lab.

4.2 Recovery

A second approach to describe performance is the percentage recovery of added analyte. In this approach the amounts of weighed quantities added to the samples are the assumed target values after adjustment for blank values. The correlation between weighed amounts (on the x-axis) and your measured quantities (on the y-axis) has been calculated. The slope of the resulting relation (a in $y = ax + b$) in this formula multiplied by 100% is your recovery of the added amounts. The outcome for your lab in comparison to the median outcome of all laboratories is shown in the column “Recovery”. The recovery is generally acceptable with 25 analytes having a recovery of between 90 - 110%. Poor recovery is evident for four analytes: 2-aminobutyric acid (65%), cystine (68%), hydroxyproline (54%) and sulfocysteine (82%).

4.3 Precision

Reproducibility is an important parameter for the analytical performance of a laboratory and is addressed in the schemes’ design. Samples provided in pairs can be regarded as duplicates from which CVs can be calculated. The column “Precision” in the annual report shows your CVs for the respective amino acids in comparison to median values for all laboratories. Precision ranges from 4.7% for methionine to 14.3% for hydroxyproline. 13 amino acids demonstrated good performance with CVs < than 6%. The average intra-laboratory CV is 6.9%.

4.4 *Linearity*

Linearity over the whole relevant analytical range is another important parameter for analytical quality and is also examined within the schemes. A comparison of the weighed quantities on the x-axis and your measured quantities on the y-axis allows calculation of the coefficient of regression (**r**). The column “Linearity” in the annual report shows your **r** values for the respective amino acids in comparison to the median **r** values for all laboratories. Ideally the **r** value is close to 1.000 and ranges from sarcosine (0.908) to 4 amino acids that give an excellent r value (r = 0.999). It must be remembered that only a limited concentration range is tested in this scheme.

4.5 Inter-laboratory CV

For comparison of amino acid levels for diagnosis and monitoring of treatment for one patient in different hospitals, and to facilitate the use of shared reference intervals, it is essential to have a high degree of harmonization. Part of the schemes’ design is to monitor this by calculating the inter-laboratory CV. This, along with the number of laboratories that submitted results, is shown in the column “Data all labs” in the annual report. Agreement between laboratories is reasonable for most amino acids, with fourteen amino acids having an inter-laboratory CV of <10%, and ten amino acids having an inter-laboratory CV between 10 and 15%. However, five amino acids have a CV >15% with argininosuccinic acid having a CV of 28.3%.

4.6 Number of Participating Laboratories and submitted results

For 22 of the individual amino acids, results were submitted in at least 258 datasets (87% of the 295 datasets).

4.7 Inter-relationships between quality parameters

The various parameters described above often have an inter-relationship: usually more than one parameter points in the same direction towards either good or bad analytical performance.
For example for alanine all parameters indicate good performance: precision (CV = 4.8%), linearity (r = 0.998), recovery (99%) and inter-lab variation (inter-lab CV 8.56) with the majority of laboratories (n=286 datasets) submitting results.

4.8 Your performance: red and green flags

In order to easily judge performance of individual laboratories, the annual report may include flags in case of poor performance for accuracy, precision, linearity and recovery. Amino acids 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 amino acid. Criteria for flags can be found in the general information on the website (on this website under general information; use website, explanation annual report).

4.9 Poor Performance Policy

A wide dispersion in the overall performance of individual laboratories is evident. Table 2 shows the percentage of red flags observed. 27% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme 5% 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 agreed on a harmonised scoring system for the various branches of the Diagnostic Proficiency schemes and qualitative schemes. The scoring system for the quantitative schemes is now well established and is 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 are 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 this will be ratified by the SAB. A letter pointing out failure to achieve these levels will be issued 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 laboratories 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 Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	5%	5%
25%	1%	6%
20 – 25%	2%	8%
15 – 20%	5%	13%
10 – 15%	6%	19%
5 – 10%	15%	34%
0 – 5%	39%	73%
0%	27%	100%

4.10 Certificates

As for other schemes, the performance, as indicated by the flags in an individual laboratory's annual report, is summarised in the annual participation certificate. The certificate lists the total number of amino acids 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.

4.11 Additional Specific Remarks of the Scientific Advisor

NA

5. Summary of performance

General comments

The results obtained this year broadly agree with what was expected. Some discrepancies with calculated recoveries are evident for a few amino acids.

Quantitative comparisons (see table 3).

The overall performance evaluated by comparing intra-laboratory variation (imprecision) with inter-laboratory variation for each amino acid reveals three main groups. There are fourteen amino acids with good intra- and inter-laboratory precision (<10%). Ten amino acids show acceptable intra- and inter-laboratory precision (CVs between 10-15) and there are five amino acids for which performance is poor, with inter-laboratory CVs > 15% (range 15-28%).

Taking all parameters into account there is a group of 22 well-established amino acids for which there is good overall performance, reflected by satisfactory values for all five analytical quality parameters (acceptable precision and inter-laboratory CV, linearity exceeding 0.9, recovery between 90 and 110%, and a high percentage of submitted results. There is also a group of seven analytes where performance is less than satisfactory; 2-aminobutyric acid; argininosuccinic acid; asparagine; citrulline; cystine; hydroxyproline; sulfocysteine.

Table 3. Summary of results of all laboratories

Analyte	Accuracy (mean µmol/L)	Precision (CV% duplicates)	Linearity (r)	Recovery (%added analyte)	Data all labs	
	All labs	All labs	All labs	All labs	n	Inter-lab CV
2-aminobutyric acid	46.6	7.3%	0.844	65%	203	11.0%
Alanine	505	4.8%	0.998	99%	286	8.56%
Alloisoleucine	36.9	9.1%	0.991	96%	219	12.8%
Arginine	325	6.0%	0.998	97%	287	9.29%
Argininosuccinic acid	39.0	12.8%	0.990	99%	149	28.3%
Asparagine	66.5	7.3%	0.989	105%	258	15.4%
Aspartic acid	61.2	6.2%	0.991	93%	268	14.7%
Citrulline	656	6.3%	0.998	97%	282	15.3%
Cystine	48.1	9.2%	0.977	68%	255	12.6%
Glutamic acid	198	6.4%	0.997	102%	283	10.2%
Glutamine	887	7.2%	0.998	98%	283	12.2%
Glycine	497	5.1%	0.998	98%	285	8.16%
Histidine	64.6	6.4%	0.987	98%	279	9.44%
Hydroxyproline	29.4	14.3%	0.775	54%	231	17.0%
Isoleucine	664	5.6%	0.999	95%	292	12.2%
Leucine	312	5.4%	0.996	100%	292	8.64%
Lysine	239	5.2%	0.998	99%	287	7.96%
Methionine	223	4.7%	0.999	97%	288	8.41%
Ornithine	495	5.5%	0.994	97%	287	7.48%
Phenylalanine	651	5.3%	0.999	94%	295	11.1%
Proline	304	6.3%	0.998	98%	272	9.77%
Sarcosine	113	8.8%	0.996	101%	151	13.4%
Serine	114	5.0%	0.998	99%	285	8.56%
Sulphocysteine	31.4	12.9%	0.986	82%	100	22.2%
Taurine	128	5.9%	0.994	101%	268	8.49%
Threonine	264	5.0%	0.997	100%	283	7.32%
Thryptophan	85.8	7.5%	0.981	90%	238	13.5%
Tyrosine	260	5.0%	0.999	96%	296	7.85%
Valine	201	4.8%	0.997	99%	294	7.32%
Overall	260	6.9%	0.981	94%	258	11.7%

Educational Effect of ERNDIM

Greater experience of amino acid analysis as reflected by longer participation in ERNDIM schemes clearly seems to contribute to improved performance. Beyond this the learning/educational effect of EQA as provided by ERNDIM is undoubtedly a major factor in improving performance.

6. Preview of the Scheme for 2024

Our policy is to include the same common amino acids in each year's samples as well as a few unusual ones which are selected year to year. The design of the 2024 scheme is essentially the same as in 2023.

7. Questions, Comments and Suggestions

If you have any questions, comments or suggestions in addition to specific user comments please address these to the scientific advisor of the scheme, Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) and/or the scheme organiser Dr. C. W. Weykamp (mca.office@skbwinterswijk.nl).

London 17/01/24

Dr. Rachel Carling
Scientific Advisor

Please note:
This annual report is intended for participants of the ERNDIM Amino Acids (serum). 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	17th January 2024	• 2023 annual report published

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