ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2022

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 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 304 datasets have been submitted, for 14 of them an annual report could not be generated due to insufficient data submission. Two 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. Eline van der Hagen 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 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.

[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 2022. 01-05**	**Sample pair 2022. 02-08**	**Sample pair 2022. 03-07**	**Sample pair 2022. 04-06**
2-aminobutyric acid	Sigma A1879	11.3	80.2	21.1	39.6
Alanine	Sigma 44526	250.9	1500.2	500.1	750.1
Alloisoleucine	Sigma I8754	5.5	99.6	11.1	50.1
Arginine	Sigma 90538	20.7	639.9	79.9	160.5
Arginino succinic acid	Sigma A5707	10.0	99.9	25.6	50.0
Asparagine	Sigma 51363	19.8	80.2	39.6	60.4
Aspartic acid	Sigma 51572	19.6	79.6	40.3	60.0
Citrulline	Sigma 1133842	25.7	1500.3	101.1	750.3
Cystine	Sigma 49603	100.3	24.8	74.9	50.1
Glutamic acid	Sigma 95436	75.0	500.2	125.3	249.6
Glutamine	Sigma 76523	250.3	1500.1	499.7	750.0
Glycine	Sigma 76524	249.3	1499.7	500.5	749.8
Histidine	Sigma 73767	19.6	80.4	39.3	59.9
Homocitrulline	Santa Cruz 269298	79.0	19.9	39.9	59.4
Hydroxyproline	Sigma PHR1939	6.6	39.9	10.0	20.4
Isoleucine	Sigma 56241	99.6	1500.1	248.9	500.2
Leucine	Sigma 76526	1496.7	250.0	998.9	500.3
Lysine	Sigma 67448	24.6	750.3	250.4	499.6
Methionine	Sigma 39496	15.6	750.0	74.9	149.6
Ornithine	Sigma O2375	49.9	749.8	249.6	500.1
Phenylalanine	Sigma 40541	25.5	1499.7	361.1	600.1
Proline	Sigma 93693	50.4	750.3	250.9	499.3
Pros-methylhistidine	Sigma M9005	79.8	19.7	60.0	40.3
Sarcosine	Sigma S7672	4.9	19.5	9.8	14.3
Serine	Sigma 54763	19.3	400.5	99.4	200.0
Sulphocysteine	Abcam Ab146303	5.2	74.8	25.5	49.8
Taurine	Sigma 93019	40.6	320.0	81.1	160.3
Tele-methylhistidine	Sigma 67520	19.7	79.8	41.2	60.0
Threonine	Sigma 61506	99.9	499.6	199.9	349.6
Thryptophan	Sigma 51145	24.9	150.0	49.8	100.3
Tyrosine	Sigma 91515	1098.1	49.7	549.5	274.7
Valine	Sigma 50848	49.5	650.1	149.8	324.2

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 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 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 of 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 32 amino acids in the year 2022 cycle, 8 x 32 = 256 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 (8 such Cycle Reviews can be requested in 2022). The Annual Report summarizes all results giving an indication of overall performance for all analytes in all 8 samples (1 such Annual-Report can be requested in 2022). Depending on the responsibilities within the laboratory, participants can choose to inspect the annual report (e.g. Quality Managers) or all (or part of) the 256 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	44.6	39.4	14.4%	8.6%	0.982	0.993	106%	100%	219	13.0%
Alanine	746	741	4.1%	4.8%	0.999	0.997	98%	97%	300	8.55%
Alloisoleucine	56.6	47.2	14.4%	8.1%	0.991	0.991	122%	95%	225	14.1%
Arginine	242	233	4.2%	6.3%	0.999	0.999	103%	99%	298	10.2%
Argininosuccinic acid		35.3		17.0%		0.984		100%	154	39.6%
Asparagine		54.4		9.0%		0.985		105%	272	20.9%
Aspartic Acid		48.9		7.7%		0.986		88%	283	17.2%
Citrulline	583	583	10.4%	6.9%	0.997	0.998	97%	98%	296	12.3%
Cystine	43.5	48.2	19.8%	8.4%	0.917	0.975	62%	64%	267	12.2%
Glutamic acid	253	247	6.4%	6.3%	0.998	0.997	108%	99%	298	10.1%
Glutamine	648	742	6.6%	6.2%	0.996	0.996	85%	98%	295	10.3%
Glycine	744	737	4.6%	5.6%	0.999	0.997	100%	96%	298	9.03%
Histidine	53.6	50.9	4.4%	7.0%	0.996	0.986	100%	91%	294	10.3%
Homocitrulline		45.9		8.6%		0.984		89%	141	14.2%
Hydroxyproline		19.8		15.7%		0.977		95%	246	21.7%
Isoleucine	500	563	5.9%	5.7%	0.999	0.999	86%	94%	306	11.4%
Leucine	773	786	13.0%	4.9%	0.987	0.997	94%	95%	308	9.30%
Lysine	390	384	4.8%	5.1%	0.999	0.998	99%	98%	302	8.63%
Methionine	ORFR	246	ORFR	6.7%	ORFR	0.999	ORFR	97%	296	11.6%
Ornithine	396	386	4.2%	6.3%	0.999	0.997	99%	98%	301	9.06%
Phenylalanine	593	595	3.0%	5.5%	1.000	0.998	93%	94%	310	9.80%
Proline		372		6.0%		0.996		98%	284	9.50%
Sarcosine		12.8		17.9%		0.908		100%	146	27.9%
Serine	183	181	7.1%	5.5%	0.998	0.998	95%	97%	300	8.55%
Sulfocysteine		32.1		15.3%		0.979		78%	96	20.8%
Taurine	154	153	2.9%	6.0%	0.999	0.998	105%	102%	279	10.0%
Threonine	298	287	6.2%	5.0%	0.995	0.996	106%	98%	298	7.80%
Tryptophan		97.2		7.0%		0.990		95%	246	13.6%
Tyrosine	465	472	12.9%	4.9%	0.993	0.999	92%	95%	311	8.99%
Valine	299	298	4.4%	4.7%	0.999	0.998	94%	99%	308	8.00%
Overall	373	285	7.7%	7.8%	0.992	0.990	97%	95%	266	13.3%

See this example of part 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 2022

In this part the results as seen in the annual report 2022 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 labs. This is shown in the columns "Your Lab" and "All Labs" under the heading "Accuracy". For example, for alanine, the mean for all labs is 741 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 labs is shown in the column “Recovery”. The recovery is generally acceptable with 26 analytes having a recovery within the range 90 - 110%. Poor recovery is evident for four analytes: aspartic acid (88%), cystine (64%), homocitrulline (89%) and sulfocysteine (78%).

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 labs. Precision ranges from 4.8% for alanine and valine to 17.9% for sarcosine. 10 amino acids demonstrated good performance with CVs < than 6%. The average intra-lab CV is 7.8%.

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 labs. 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-lab CV

For comparison of amino acid levels for diagnosis and monitoring of treatment for one patient in different hospitals and for use of shared reference values it is essential to have a high degree of harmonization between results of laboratories. 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 eleven amino acids having an inter lab CV of <10% and thirteen between 10 and 15%. However, six amino acids have a CV >15% with argininosuccinic acid having a CV of 39.5%.

4.6 Number of Participating Labs and submitted results

For 22 of the individual amino acids, results were submitted in at least 266 datasets (88% of the 304 datasets).

4.7 Inter-relationships between quality parameters

The various parameters described above often have an interrelationship: 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 = 0.997), recovery (97%) and inter-lab variation (inter-lab CV 8.55) with the majority of labs (n=300 datasets) submitting results.

4.8 Your performance: red and green 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. 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. 30% of the laboratories have no flag at all and thus have attained excellent overall performance. In contrast, at the other extreme 7% 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 labs in the pursuit of our overall aim to improve quality of diagnostic services in this field.

Table 2. Percentage Red Flags

% Red Flags seen in Annual Report	Percentage Labs In this Category	Cumulative Percentage Of Labs
>25%	7%	7%
25%	1%	8%
20 – 25%	1%	9%
15 – 20%	3%	12%
10 – 15%	8%	20%
5 – 10%	18%	38%
0 – 5%	38%	76%
0%	24%	100%

4.10 Certificates

As for other schemes, the performance, as indicated by the flags in the individual laboratories 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

Pros and Tele methylhistidine, homocitrulline and sarcosine were present in the eight 2022 samples but were not included in the annual report. Sarcosine was included to increase awareness of the potential to interfere with alanine when measured by LC-MS/MS. Likewise, homocitrulline was included to raise awareness of the potential for it to interfere with methionine when measured by IEC. Pros and tele methylhistidine were included to raise awareness of the change in nomenclature of these two compounds. Education is a key aspect of the ERNDIM scheme.

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-lab variation (precision) with inter-lab variation for each amino acid reveals three main groups. There are twelve amino acids with good intra and inter-lab precision (<10%). Eleven amino acids show acceptable intra and inter-lab precision (CVs between 10-15) and there are five amino acids for which performance is poor, with inter-lab CV > 15% (range 17-40%).

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-lab CV, linearity exceeding 0.9, recovery between 90 and 110% and a high percentage of submitted results. There is also a group of six analytes, argininosuccinic acid, asparagine, aspartic acid, cysteine, hydroxyproline and sulfocysteine, where performance is less than satisfactory.

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	39.4	8.6%	0.993	100%	219	13.0%
Alanine	741	4.8%	0.997	97%	300	8.55%
Alloisoleucine	47.2	8.1%	0.991	95%	225	14.1%
Arginine	233	6.3%	0.999	99%	298	10.2%
Arginino succinic acid	35.3	17.0%	0.984	100%	154	39.6%
Asparagine	54.4	9.0%	0.985	105%	272	20.9%
Aspartic acid	48.9	7.7%	0.986	88%	283	17.2%
Citrulline	583	6.9%	0.998	98%	296	12.3%
Cystine	48.2	8.4%	0.975	64%	267	12.2%
Glutamic acid	247	6.3%	0.997	99%	298	10.1%
Glutamine	742	6.2%	0.996	98%	295	10.3%
Glycine	737	5.6%	0.997	96%	298	9.03%
Histidine	50.9	7.0%	0.986	91%	294	10.3%
Hydroxyproline	19.8	15.7%	0.977	95%	246	21.7%
Isoleucine	563	5.7%	0.999	94%	306	11.4%
Leucine	786	4.9%	0.997	95%	308	9.30%
Lysine	384	5.1%	0.998	98%	302	8.63%
Methionine	246	6.7%	0.999	97%	296	11.6%
Ornithine	386	6.3%	0.997	98%	301	9.06%
Phenylalanine	595	5.5%	0.998	94%	310	9.80%
Proline	372	6.0%	0.996	98%	284	9.50%
Serine	181	5.5%	0.998	97%	300	8.55%
Sulphocysteine	32.1	15.3%	0.979	78%	96	20.8%
Taurine	153	6.0%	0.998	102%	279	10.0%
Threonine	287	5.0%	0.996	98%	298	7.80%
Thryptophan	97.2	7.0%	0.990	95%	246	13.6%
Tyrosine	472	4.9%	0.999	95%	311	8.99%
Valine	298	4.7%	0.998	99%	308	8.00%
Overall	285	7.8%	0.990	95%	266	13.3%

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 2023

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 2023 scheme is essentially the same as in 2022.

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. Eline van der Hagen (mca.office@skbwinterswijk.nl).

London 13/01/23

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	13th January 2023	• 2022 annual report published

END