ERNDIM Quantitative Schemes Amino Acids(serum)

ANNUAL REPORT 2024

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

3. *Design*

The scheme has been designed, planned and coordinated by the scientific advisors, Dr. Rachel Carling and Dr. Zoe Barclay, 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 distributes the 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 2024. 01-08**	**Sample pair 2024. 02-06**	**Sample pair 2024. 03-05**	**Sample pair 2024. 04-07**
2-aminobutyric acid	Sigma A1879	74.6	25.5	51.0	5.8
Alanine	Sigma 44526	101	598	300	900
Alloisoleucine	Sigma I8754	2.2	25.3	10.1	49.8
Arginine	Sigma 90538	20.1	200	80.0	600
Argininosuccinic acid	Sigma A5707	15.5	60.1	30.0	120
Asparagine	Sigma 51363	15.4	91.0	30.1	119
Aspartic acid	Sigma 51572	250	50.2	126	25.1
Citrulline	Sigma 1122842	1000	200	596	19.9
Cystine	Sigma 49603	20.2	60.0	40.0	79.8
Glutamic acid	Sigma 95436	300	99.9	150	50.6
Glutamine	Sigma 76523	1490	500	1001	49.7
Glycine	Sigma 76524	98.6	601	280	900
Histidine	Sigma 73767	29.8	120	59.8	241
Hydroxyproline	Sigma PHR1939	150	50.4	100	25.4
Isoleucine	Sigma 56241	60.0	359	180	720
Leucine	Sigma 76526	49.6	500	150	1000
Lysine	Sigma 67448	20.5	200	100	299
Methionine	Sigma 39496	8.9	299	100	600
Ornithine	Sigma O2375	650	150	326	74.9
Phenylalanine	Sigma 40541	36.7	600	360	1200
Proline	Sigma 93693	50.2	150	99.7	300
Sarcosine	Sigma S7672	10.6	39.9	19.9	79.7
Serine	Sigma 54763	36.7	155	72.4	307
Sulphocysteine	TRC-S789483	9.5	75.0	24.9	99.9
Taurine	Sigma 93019	361	121	240	60.4
Threonine	Sigma 61506	25.5	126	50.1	250
Tryptophan	Sigma 51145	10.1	39.7	20.1	80.3
Tyrosine	Sigma 91515	901	300	600	100
Valine	Sigma 50848	49.7	298	100	601

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 and the request and viewing of reports, proceed 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 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 a range of analytical parameters (accuracy, precision, linearity, recovery and inter-lab dispersion) to be reported once the annual cycle has been completed.

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 2024 cycle, 232 (8 x 29) 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 2024). The Annual Report summarises 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	34.5	39.3	4.7%	6.6%	0.999	0.996	88%	102%	190	10.8%
Alanine	476	478	1.4%	4.5%	0.997	0.998	107%	99%	280	7.68%
Alloisoleucine	MP	22.3	3.6%	10.2%	0.997	0.995	113%	101%	213	16.5%
Arginine	229	238	6.2%	5.3%	0.998	0.999	100%	100%	281	9.41%
Argininosuccinic acid	44.0	38.9	16.0%	11.8%	0.987	0.989	96%	81%	152	29.8%
Asparagine	77.8	62.9	11.9%	8.3%	0.984	0.993	141%	97%	253	20.1%
Aspartic Acid	88.0	111	3.5%	7.7%	0.998	0.995	68%	88%	263	20.0%
Citrulline	422	448	5.7%	6.0%	0.998	0.998	91%	98%	277	11.3%
Cystine	32.5	36.1	5.3%	8.4%	0.991	0.985	61%	67%	248	11.8%
Glutamic acid	151	166	9.4%	6.0%	0.996	0.996	98%	107%	278	10.1%
Glutamine	674	752	4.4%	6.9%	0.999	0.997	87%	99%	277	11.2%
Glycine	467	468	2.6%	4.5%	0.997	0.998	104%	98%	279	7.70%
Histidine	106	110	2.6%	5.6%	0.999	0.998	98%	94%	273	9.81%
Hydroxyproline	68.6	80.9	10.5%	9.6%	0.997	0.990	95%	99%	228	14.1%
Isoleucine	345	324	11.2%	4.4%	0.990	0.999	119%	98%	283	7.99%
Leucine	454	420	18.8%	4.5%	0.982	0.999	118%	97%	285	8.78%
Lysine	160	161	3.1%	5.2%	0.997	0.998	107%	100%	281	8.03%
Methionine	245	248	1.7%	4.9%	0.999	0.999	101%	98%	286	8.67%
Ornithine	278	302	6.3%	5.2%	0.998	0.998	88%	98%	279	9.35%
Phenylalanine	504	529	3.0%	4.3%	0.999	0.999	95%	95%	288	7.60%
Proline	140	150	8.0%	5.9%	0.994	0.997	98%	100%	268	9.28%
Sarcosine		37.3		10.4%		0.993		99%	146	16.0%
Serine	140	142	3.4%	5.0%	0.998	0.998	103%	99%	278	7.94%
Sulfocysteine		33.1		11.7%		0.987		61%	103	22.5%
Taurine	179	198	7.2%	5.1%	0.996	0.997	93%	101%	263	9.90%
Threonine	113	115	2.9%	5.2%	0.998	0.998	104%	100%	277	8.63%
Tryptophan	45.8	61.4	14.9%	6.9%	0.960	0.989	67%	94%	235	13.6%
Tyrosine	415	461	6.5%	5.1%	0.997	0.997	85%	96%	289	7.60%
Valine	260	264	1.2%	4.6%	0.999	0.999	103%	98%	287	8.05%
Overall	237	224	6.5%	6.6%	0.994	0.996	97%	95%	253	11.9%

Above is an example of an annual report. 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 2024

In this part the results as seen in the annual report 2024 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 478 micromol/litre. with which you can compare the mean of your lab.

It is important to recognise that using ERNDIM QTAS EQA material to establish bias is potentially a limitation. The bias of the method has been determined by comparing results to a derivation of the ERNDIM all laboratory trimmed mean, not a true target value. As such, the bias determined is not a measure of absolute accuracy and is simply a measure of performance relative to other laboratories.

4.2 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 4.3% for phenylalanine to 13.4% for sulphocysteine. 16 amino acids demonstrated good performance with CVs < than 6%. The average intra-laboratory CV is 6.7%.

4.3 *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 added 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 cystine (0.984) to 6 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.

4.4 *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 94 - 107%. Poor recovery is evident for four analytes: argininosuccinic acid (81%), aspartic acid (88%), cystine (67%), and sulphocysteine (62%).

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 harmonisation. Part of the schemes' design is to monitor this by calculating the inter-laboratory variation. 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 16 amino acids having an inter-laboratory CV of <10%and seven amino acids having an inter-laboratory CV between 10 and 15%. However, six amino acids have a CV >15% with argininosuccinic acid having the greatest CV, at 29.8%.

4.6 Number of Participating Laboratories and submitted results

For 20 of the individual amino acids results were submitted in at least 258 datasets (90% 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 = 4.5%). linearity (r = 0.998). recovery (99%) and inter-lab variation (inter-lab CV 7.70%) with the majority of laboratories (n=280 datasets) submitting results.

4.8 Your performance: 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; interactive 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. 34% 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. However, it should be noted that not all laboratories return results for all analytes. Intensive discussion within the Scientific Advisory Board (SAB) resulted in a harmonised scoring scheme that has been in place for the quantitative schemes for more than ten years. Likewise, there has been agreement as to what constitutes satisfactory performance. Both parameters are checked annually and if necessary re-evaluated. 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%	2%	7%
20 – 25%	3%	10%
15 – 20%	5%	15%
10 – 15%	7%	22%
5 – 10%	16%	38%
0 – 5%	28%	66%
0%	34%	100%

4.10 Certificates

Overall performance (as indicated by red/green flags in each 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 should be viewed in conjunction with the individual annual report in the case of internal or external auditing.

4.11 Additional Specific Remarks of the Scientific Advisor

The scheme results are broadly consistent with those seen in previous years, with alloisoleucine, argininosuccinic Acid, asparagine, aspartic Acid, cystine, and sulphocysteine highlighted as poorer performing analytes. Additionally sarcosine (added to increase the awareness of the potential to intefere with LC-MS/MS analysis of alanine) was noted to have high intra- and inter-lab imprecision (10.5% and 16.3% respectively).

Other analytes generally demonstrated good overall performance, reflected by satisfactory values for all five analytical quality parameters (intra-lab imprecision ≤ 9.6%, linearity (with the exception of tryptophan) > 0.990, recovery of 94% - 107% and inter-lab imprecision ≤ 13.7%).

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 sixteen amino acids with good intra- and inter-laboratory precision (<10%). Seven amino acids show acceptable intra- and inter-laboratory precision (intra-lab precision <10% and inter-lab precision between 10-15) and there are six amino acids for which performance is poor, with inter-laboratory CVs > 15% (range 16-30%).

Taking all parameters into account there is a group of 23 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 six analytes where performance is less than satisfactory; allo-isoleucine; argininosuccinic acid; asparagine; aspartic acid; cystine; sulphocysteine.

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	39.3	6.6%	0.996	102%	190	10.8%
Alanine	478	4.5%	0.998	99%	280	7.70%
Alloisoleucine	22.3	10.2%	0.995	101%	214	16.6%
Arginine	238	5.3%	0.999	100%	281	9.41%
Argininosuccinic acid	38.9	12.5%	0.989	81%	153	29.8%
Asparagine	63.0	8.5%	0.992	97%	253	20.4%
Aspartic acid	111	7.9%	0.995	88%	263	20.0%
Citrulline	448	6.0%	0.998	98%	277	11.3%
Cystine	36.1	8.7%	0.984	67%	248	11.9%
Glutamic acid	166	6.0%	0.996	107%	278	10.1%
Glutamine	752	6.9%	0.997	99%	277	11.2%
Glycine	468	4.5%	0.998	98%	279	7.70%
Histidine	110	5.6%	0.998	94%	273	9.82%
Hydroxyproline	81.1	9.6%	0.990	99%	228	14.1%
Isoleucine	324	4.4%	0.999	98%	283	8.01%
Leucine	420	4.5%	0.999	97%	285	8.80%
Lysine	161	5.2%	0.997	100%	281	8.09%
Methionine	248	4.9%	0.999	98%	286	8.71%
Ornithine	302	5.2%	0.998	98%	279	9.36%
Phenylalanine	529	4.3%	0.999	95%	288	7.61%
Proline	150	5.9%	0.997	100%	268	9.37%
Sarcosine	37.3	10.5%	0.993	99%	146	16.3%
Serine	142	5.0%	0.998	99%	278	7.97%
Sulphocysteine	33.3	13.4%	0.985	62%	104	22.6%
Taurine	198	5.1%	0.997	101%	263	9.91%
Threonine	115	5.2%	0.998	100%	277	8.62%
Thryptophan	61.6	6.9%	0.989	94%	235	13.7%
Tyrosine	461	5.1%	0.997	96%	289	7.61%
Valine	264	4.6%	0.999	98%	287	8.06%
Mean	224	6.7%	0.995	95%	253	11.9%

6. Preview of the Scheme for 2025

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 2025 scheme is essentially the same as in 2024.

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 advisors of the scheme. Dr. Rachel Carling (Rachel.Carling@viapath.co.uk) and Dr. Zoe Barclay, and/or the scheme organiser Dr. C. W. Weykamp (mca.office@skbwinterswijk.nl).

London, 16/12/24

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	20th January 2025	• 2024 annual report published

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